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PART II. MAMMEIGIN: A NEW NATURALLY OCCURRING 4-PHENYLCOUMARIN.

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PART I

THE ENDECAPHYLINS;
NATURALLY OCCURRING ALIPHATIC NITRO-COMPOUNDS

PART II

MAMMEIGIN;
A NEW NATURALLY OCCURRING 4-PHENYLCOUMARIN

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Wolfgang Heinz Mueller

* * * * *

The Ohio State University
1964

Approved by

[Signature]
Adviser
Department of Chemistry
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PUBLICATION

Naturally Occurring Aliphatic Nitro-Compounds; The
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ABSTRACT

Part I.- From *Indigofera endecaphylla* Jacq. ethyl- 
\(\beta\)-nitropropionate, \(\beta\)-nitropropionic acid, succinic acid, 
\(\beta\)-methyl-D-glucoside, and eleven aliphatic nitro-com-
phounds, the endecaphyllins, were isolated. Six of the
endecaphyllins were shown to be isomeric tris-\(O-\)\(\beta\)-nitro-
propionyl)-glucosides and three of them isomeric di-\(O-\)
(\(\beta\)-nitropropionyl)-glucosides. Furthermore, there was
obtained a tetra-\(O-\)\(\beta\)-nitropropionyl)-glucoside, together
with a glucose diester of a presumed nitrovaleric acid.
Structural elucidation of the positional isomers was
attempted. Synthetic methods for \(O-\beta\)-nitropropionyl-
glucosides were investigated. Tetra-\(O-\)\(\beta\)-nitropropionyl-
\(\beta\)-methyl-D-glucoside, a tris-\(O-\)\(\beta\)-nitropropionyl)-\(\beta\)-
methyl-D-glucoside, and a tetra-\(O-\)\(\beta\)-nitropropionyl)-
glucose derivative were synthesized.

Part II.- A new 4-phenylcoumarin, mamegin, was
isolated from *Mamnea americana* L. It was shown to be
5-hydroxy-6-isovaleryl-8,8-dimethyl-4-phenyl-2\(H\),8\(H\)-benzo
[\(1,2-b : 3,4-b'\)]dipyran-2-one. The n.m.r. spectra of
mamegin, its dihydro derivative, and mameisin(4-phenyl-
5,7-dihydroxy-6-isovaleryl-8-isopentenylcoumarin), are
discussed.
HISTORICAL REVIEW

Naturally Occurring Nitro-Compounds.

Aromatic Nitro-Compounds.

Until the present day only a few naturally occurring nitro-compounds have been described. In 1949 the first compound, chloramphenicol (1), an antibiotic metabolite produced by cultures of the soil bacterium Streptomyces venezuelae, was characterized.

\[
\begin{align*}
0_2N & \quad \text{(1)} \\
\text{OH} & \quad \text{NH-CO-CHCl}_2 \\
\text{CH-CH-CH}_2\text{OH} & 
\end{align*}
\]

From an unidentified streptomyces species, the antibiotic substance azomycin (2) was obtained.

\[
\begin{align*}
\text{HN} & \quad \text{(2)} \\
\text{NO}_2 & 
\end{align*}
\]

The aristolochic acids 3 and 4, obtainable from seeds of Aristolochia clematitis, roots of A. longa L., A. rotunda L., and A. argentina Griseb., were recognized only a few years ago and their structures elucidated. As long ago as the Greco-Roman period,
various species of Aristolochia were used in therapeutics. In test animals these acids cause cardiac and respiratory arrest. Aristolochic acids, isolated from *A. indica*, showed reproducible anticancer activity against the standard laboratory type of mouse cancer.\(^{10}\) Aureothin (5),\(^{11}\) a by-product of the antibiotic aureothricin, is a toxin isolated from the culture of *Streptomyces thioluteus*.\(^{12}\) Besides the rare function of a nitro-group, this compound shows also the structural peculiarity of a 2-methoxy-\(\delta\)-pyrone which had not previously been found in nature. All the other naturally occurring 2,4 pyrones such as yangonin, anibine, 4-methoxyparacotoxin, and 5,6-dehydrokavain are 4-methoxy-
α-pyrones.

**Aliphatic Nitro-Compounds.**

Of the aliphatic class of nitro-compounds, β-nitropropionic acid,13-19 l-nitro-2-phenylethane,20 hiptagin,21 and karakin22,23 appear to be the only naturally occurring representatives which have, until now, been reported.7

**β-Nitropropionic Acid.**—This acid has been isolated from *Indigofera endecaphylla* Jacq. (family of Leguminosae),13 and from roots of *Viola odorata* (family of Violaceae).15 It is of interest that β-nitropropionic acid is also a mould metabolite. On purification of flavicin, a substance similar to penicillin, which is produced by *Aspergillus flavus* cultures, 900 mg. of β-nitropropionic acid per 90 l. of culture medium was found.16,17 β-Nitropropionic acid was also isolated together with a new antibiotic, oryzacidin, from cultures of *Aspergillus oryzae*.18

In order to obtain some information about the biochemistry of these micro organisms, studies with *Penicillium atrovenetum* G. Smith were conducted.19 *P. atrovenetum* grown on Raulin-Thom medium (consisting of mineral salts, sucrose, tartaric acid, and ammonium salts), produces β-nitropropionic acid (2.4 g./l. of culture medium) as a major metabolite. Surprisingly
enough, the yield of $\beta$-nitropropionic acid is only 10% when Czapek-Dox solution, which contains sodium nitrite as a sole nitrogen source, is used instead of Baulin-Thom solution. It was also found that the production of $\beta$-nitropropionic acid increases proportionally with the development of the mould and decreases towards the end of the growth. In addition, 65% of the ammonia-nitrogen consumed by the fungi became incorporated in the $\beta$-nitropropionic acid. When 2-$^{14}$C-$\beta$-alanine (6), NaH$^{14}$CO$_3$, or 4-$^{14}$C(4)-aspartic acid (7) was fed to P. atrovenetum, it was found that only from the last two substrates radioactivity was incorporated in the resultant $\beta$-nitropropionic acid (8). Incorporation from NaH$^{14}$CO$_3$ showed 0.04% radioactivity with 97% label located on 1-position and 1% on the 2-position. From the labeled aspartic acid, 2% of the radioactivity was found in the $\beta$-nitropropionic acid (96% on C$_1$, none on C$_2$). The authors concluded therefore, that the incorporation of aspartic acid into $\beta$-nitropropionic acid via oxidation and decarboxylation of the nitro-acid (9) took place. In
This connection it is also interesting, that the only other aliphatic representative is 1-nitro-2-phenylethane (10), which could conceivably arise from phenylalanine (11) by a similar oxidation of the amino group and decarboxylation.

\[
\text{C}_6\text{H}_5\text{CH}_2\text{OH} - \text{CO}_2\text{H} \quad \text{(11)} \xrightarrow{[0]} \quad \text{C}_6\text{H}_5\text{CH}_2\text{NO}_2 \text{CO}_2\text{H} \quad \text{(10)}
\]

**1-Nitro-2-phenylethane.** - This compound was found to be responsible for the cinnamon odor of the brazilian trees *Ocotea pretiosa* (Nees) Mez and *Aniba canellilla* (H. B. K.) Mez (family of Lauracea). It was isolated\(^{26}\) from *Ocotea pretiosa* wood (yield 0.1%) and bark, as well as from *Aniba canellilla* wood (yield 0.7%) and bark (yield 0.6%).

**Hiptagin.** - A nitro-compound of yet unknown structure, was first isolated\(^{25}\) from the roots of *Hiptage madablotata* Gaertn. (family of Malpighiaceae). The natives of Java attributed some aphrodisiac properties to a concoction of the roots. However, detailed instructions about periodical dispensation is reported
only for bulls. Extracting the bark of the roots with refluxing acetone provided in 4-8% yield a crystalline compound, m. p. 110°. Gorter found, during more extensive investigations, that 1% of this compound also occurred in the bark of the branches. In addition, the yield obtained from the roots was increased by an improved isolation procedure. Repeated microanalyses and the observation that one half mole of water was lost in vacuo over phosphorous pentoxide at 110°, led to the empirical formula C_{10}H_{14}O_{9}N_{2}\cdot1/2 H_{2}O.

In view of the similarity of the chemical and physical properties of hiptagin with those of the endecaphyllins, some of its chemistry is of particular interest. Hiptagin was found to reduce Fehling's solution and silver salts in ammonium hydroxide. It did not form a picrate. When it was treated with hot aqueous alkali, ammonia was liberated. Upon fast acidification of the basic solution hydrogen cyanide was evolved. In hot diluted sulfuric acid hiptagin formed glucose. The equation for the hydrolysis was balanced with an assumed compound hiptagenin (C_{4}H_{4}O_{6}N_{2}). This compound was considered to be very unstable because its existence could not be substantiated by isolation.

\[
C_{10}H_{14}O_{9}N_{2} + H_{2}SO_{4} \rightarrow C_{6}H_{12}O_{6} + C_{4}H_{4}O_{6}N_{2}
\]
However, hydrolysis of hiptagin in 5% sulfuric acid, heated in a sealed tube on a steambath, yielded 57% glucose and tartronic acid \((\text{HO-CH-(CO}_2\text{H})_2\)) in unreported yield. The following formula \((12)\) for hiptagin was proposed:

\[
\begin{align*}
\text{HO-NH-CO} & \quad (12) \\
\text{NC-CH-CO-C=NOH} & \quad (14) \\
\text{NC-CH-CO-C=NOH} & \quad (15)
\end{align*}
\]

The "mysterious" compound hiptagenin was considered to be an isoxazole \((12)\), which might isomerize under the influence of acid or base to \((14)\) or \((15)\), and thus help to account for the hydrolysis products.
Enzymatic hydrolysis with emulsin, which had been proven to be specific for \( \beta \)-glucosides failed. This, and the positive rotation led the author to assume that he was dealing with an \( \alpha \)-glucoside.

When 10 g. of hiptagin was hydrolyzed in acetone and aqueous hydrochloric acid, 3 g. of a new compound melting at 68° was obtained. From its microanalytical data the formula \( \text{C}_3\text{H}_5\text{O}_4\text{N} \) was derived. In the same reaction carbon dioxide and ammonia were detected. Dry distillation of hiptagin yielded the same new compound which was called hiptagenic acid. On treating hiptagenic acid with hot aqueous alkali, ammonia was evolved, and on acidification of the reaction mixture hydrogen cyanide, formic acid, oxalic acid, and carbon dioxide were found. In aqueous hydrogen chloride, however, malic acid, formic acid, and hydroxylamine were obtained. Several formulae\(^{21,23(b)} \) were proposed for hiptagenic acid, but eventually it was identified as \( \beta \)-nitropropionic acid by comparison with a sample synthesized from \( \beta \)-iodopropionic acid and silver nitrite.\(^ {26} \) \( \beta \)-Nitropropionic anhydride,\(^ {23(b)} \) m. p. 70°, was obtained when \( \beta \)-nitropropionic acid was refluxed with acetylchloride for 2 hrs. Treating ethyl-\( \beta \)-nitropropionate with 10% aqueous ammonia yielded the corresponding amide.\(^ {23(b)} \)
In connection with the complexity of the product mixture obtained when hiptagin and \( \beta \)-nitropropionic acid are hydrolized, it is of interest to consider the hydrolysis of \( 1 \)-nitro-2-phenylethane.\(^{20}\) On heating in 3\% aqueous sodium hydroxide for 30 min., or in 1 N alcoholic potassium hydroxide for 60 min., benzoic acid and hydrocyanic acid were obtained in 15\% and 20\% yield respectively. The authors proposed the following mechanism and were able to provide some experimental support e.g.,

\[
\begin{align*}
\text{C}_6\text{H}_5\text{-CH}_2\text{-CH}_2\text{-NO}_2 & \quad \rightarrow \quad \text{C}_6\text{H}_5\text{-CH}\text{-CH-N}^+\text{OH}^- \quad \downarrow \text{OH}^- \\
\text{C}_6\text{H}_5\text{-OH}=\text{CH-N}=\text{O} & \quad \rightarrow \quad \text{C}_6\text{H}_5\text{-CH}\text{-CH-N}=\text{O} \quad \text{[\(6\)]} \quad \rightarrow \quad \text{C}_6\text{H}_5\text{-CHO} + \text{CH}_2\text{-N}=\text{OH} \quad \text{[\(-\text{H}_2\text{O}\)]} \quad \downarrow \text{H}_2\text{O} \quad \text{HCON}
\end{align*}
\]
Applying this mechanism to $\beta$-nitropropionic acid, one can readily account for the reported products obtained on hydrolysis of hiptagin and $\beta$-nitropropionic acid e.g.,

$$\begin{align*}
O_2N-CH_2-CH_2-CO_2H & \rightleftharpoons \text{HO-N=CH-CH}_2-CO_2H \\
& \downarrow \\
O=N-CH=CH-CO_2H & \rightarrow \text{HO-N=CH-CH-CO}_2H
\end{align*}$$

Qualitative support for this hydrolysis scheme was obtained in the present study by the detection of ammonia on vigorous base treatment of $\beta$-nitropropionic acid.
Karakin. - The karaka (Corynocarpus loevigata) is a New Zealand berry producing tree. The cooked kernels were used by Maoris for food. When raw, they contain a poisonous principle called karakin, which when first isolated\(^2\) from the minced kernels by extraction with cold ethanol, the formula \((C_5H_8O_5N)_3\) was assigned. The white solid melting at 122° gave after hydrolysis with hydrochloric acid an osazone with phenylhydrazine,\(^2\) m.p. 202°, and was therefore regarded as a glucoside. Hydrolysis in boiling water\(^{23(b)}\) yielded two equivalents of \(\beta\)-nitropropionic acid, and a residue which was erroneously considered an aminohexose. More systematic investigations by Carter\(^{22}\) showed one molecule of glucose per molecule of karakin. A solution of karakin in acetone and hydrochloric acid, at room temperature for two weeks, yielded three equivalents of \(\beta\)-nitropropionic acid. In 10% aqueous ammonia\(^{23(b)}\) karakin gives a bright yellow solution which is strongly dextro-rotatory, \([\alpha]_D^{20} = +133°\). From this solution \(\beta\)-nitropropionamide, m. p. 98°, was isolated. This strongly indicated an ester structure for karakin, the constitution of which was shown to be most likely 1,4,6-tris-O-(\(\beta\)-nitropropionyl)-D-glucopyranose\(^{16}\). The following arguments supported this structure assign-
acetylation of karakin gave an almost quantitative yield of diacetylkarkin, m.p. 103°.

\[
\begin{align*}
\text{HO} & \quad \text{CO-CH}_2\text{-CH}_2\text{-NO}_2 \\
\text{O}_2\text{N-CH}_2\text{-CH}_2\text{-CO-O} & \quad \text{H} \\
\text{H} & \quad \text{HO} \\
\text{H} & \quad \text{H} \\
\end{align*}
\]

On hydrolysis of karakin in boiling water, carbon dioxide was liberated, and \(\beta\)-nitropropionic acid was extracted with ether. The sugar moiety was separated by partition chromatography on cellulose with n-butanol-acetic acid-water as solvent. The first compound eluted, crystalline needles, m.p. 138°, turned out to be a bis-0-(\(\beta\)-nitropropionyl)-glucose. The second compound, a syrup, yielded \(\beta\)-nitropropionic acid on hydrolysis and was therefore considered to be a mixture of two isomeric mono-0-(\(\beta\)-nitropropionyl)-glucoses. The third component was crystalline D-glucose. The following experimental findings demonstrated the relative positions of the three acyl groupings in karakin. Abortive
attempts to prepare a trityl derivative indicated the location of one acyl group on C6. Another acyl group was placed at C1 because the compound was non-reducing. The bis-0-(β-nitropropionyl)-glucose, m.p. 138°, formed by partial hydrolysis of karakin, is reducing and consumes two moles of periodate. This showed that there are three adjacent hydroxyl groups which have to be at C1, C2, and C3. It follows that the third acyl group is located at C4 or C5. However, assuming a pyranose form this excludes C5. The low specific rotation, \([\alpha]_D^{20} = +4.5°\), indicates that it is a β-glucose derivative. Methylation attempts yielded only hydrolytic products as the glucoside is very susceptible towards alkali. Attempts to prepare acetonide or benzylidene derivatives were also unsuccessful. Hydrogenation of karakin using Adams' catalyst or Raney nickel proved futile.

**Indigofera Endecaphylla.**

Several Indigofera species have been used as forage or cover crops in the Orient. One species, *Indigofera endecaphylla* Jacq. (trailing or creeping indigo) was introduced or occurred naturally in Hawaii,
Puerto Rico, Ceylon and other tropical countries. It is a prostrate plant which makes second growth when cut. By the first frost of the season the leaves are killed, however, growth is resumed from the stems in spring, which usually obtain a height of 6 to 9 inches. In view of very promising results obtained in tests of relative palatability on a group of eleven tropical legume species, I. endecaphylla was regarded with interest as a potential pasture legume. Using dairy cattle as test animals, I. endecaphylla was found to be the outstanding species in respect to consumption and preference. But several years after the plant had been introduced into Hawaii, severe toxic symptoms were observed in herds of dairy cattle. This initiated a breeding program aimed at producing a nontoxic species, which appears to be the only possibility to utilize the outstanding agronomic characteristics of I. endecaphylla.

Various systematic studies of its toxic effect on cows, heifers, sheep, and rabbits are reported. Frequent abortion or stillbirth in pregnant cows or heifers was caused when I. endecaphylla was used as pasturage. The calves were born prematurely by as much as 100 days. Feeding tests with the legume mixed with
grasses, harvested, or chopped, in a fresh or semi-dry state, afforded no change in toxicity. Other symptoms caused were anorexia, loss of weight, and emaciation. In advanced cases a motor nerve disturbance became evident e.g., heifers walked in circles, sheep pressed their heads against the fence. In sheep and rabbits a serous discharge from the eyes was observed. Some of the sheep died after 28 days, while others became so weak that euthanasia was performed. Rabbits died within 7 to 30 days. The most noticeable effect on vital organs was on the liver, however, disturbances in the heart, kidneys, and lungs were also noted. In the liver, congestion, fatty degeneration, and cirrhosis occurred. Surprisingly enough, extended feeding tests with guinea pigs showed that these animals were able to survive for longer periods (2 years) on a diet in which I. endecaphylla constituted the only green forage. The test animals maintained normal weight and showed no toxic symptoms except that pregnant females invariably aborted. $\beta$-nitropropionic acid, the only isolated and identified crystalline compound, was considered to be the toxic constituent of I. endecaphylla. The results, however, stem from essays on chicks, which were described as convenient and reliable test animals.
for routine checks for toxicity of exotic legumes. If toxic, the chicks grew more slowly and exhibited symptoms of paralysis of the neck, legs and wings. These characteristics, as well as death, became manifest within 21 days.

Very little is known about the mode of action of aliphatic nitro-compounds in general or the metabolism of \( \beta \)-nitropropionic acid per se. It was demonstrated that nitroethane, which is relatively nontoxic, is oxidized in animals to acetaldehyde and nitrite, the latter appearing in the blood. In the case of \( \beta \)-nitropropionic acid, however, where the acid was found in the brain tissue of animals exhibiting convulsive motions, paralysis, and collapse, it is believed to be an inhibitor of an enzyme system at a site that controls muscular movement. When administered in sublethal dosage it is metabolized by the removal of the nitro group from the carbon chain. Excretion is not a major mode of detoxication.

Severe liver damage has been induced in mice by feeding the seed of *I. endecaphylla*.\(^{33(a)}\) Heating the seeds strongly did not reduce the toxicity. A chloroform-extracted residue of leaves which was free of \( \beta \)-nitropropionic acid caused similar liver damage.
Also green leaves, dried leaves or seeds of I. endecaphylla when fed to rabbits developed the same damage. No $\beta$-nitropropionic acid could be detected in the seed, however, it is present in the leaf. Three strains with quite different content of $\beta$-nitropropionic acid caused the same degree of liver damage. In addition, synthetic $\beta$-nitropropionic acid showed no toxic effects on rabbits when force fed in amounts comparable to that occurring in the plant. It appears, therefore, that the main toxin of I. endecaphylla is not $\beta$-nitropropionic acid. This is also supported by Coleman et al. who found that, in toxic fractions obtained by cation-exchange resin columns, the amount of toxic principle in the herbage appears to be less than in the seed.

The reports suggesting the existence of toxins other than $\beta$-nitropropionic acid in this plant, prompted the present investigation of I. endecaphylla.
RESULTS AND DISCUSSION

Isolation.

A crude toxic acetone extract of the leaves and stems of \textit{I. endecaphylla} was kindly supplied by Dr. M. P. Morris. Initial attempts at column chromatography on cellulose powder, aluminum oxide, and silica gel proved the latter to be the most promising. In these preliminary experiments, only oily fractions were obtained. Nevertheless, evidence that some separation had occurred was obtained by paper chromatographic comparison of the individual fractions with the crude extract.

Application of micromethods like paper and thin layer chromatography was very limited, since no solvent system was found which was satisfactory in separating the components sufficiently well.

Very careful chromatography on a silica gel column with unusually small increases of ethanol in water saturated chloroform provided the compounds listed in the order of elution in Table I.

The endecaphyllins must be present in much larger amounts than indicated by the yields reported in Table I. Since the chromatography was not carried out in a thermostated room and not run continuously,
the overlap of the individual endecaphyllins results in relatively large intermediate oily fractions, the infrared spectra of which exhibited the same characteristics as the ones of the pure endecaphyllins. Furthermore, it was noticed that acetone or ethanol solutions of the pure compounds gradually turn yellow. The crystalline solids, however, are stable for indefinite periods.

When the leaves and stems of *I. endecaphylla* were extracted consecutively with Skelly Solve B, chloroform, and ethanol, the infrared spectrum of the chloroform extract showed only a very weak nitro band at 1550 cm\(^{-1}\), whereas the ethanol extract exhibited a much stronger one. The Skelly Solve B extract showed no nitro group at all in its infrared spectrum. Ethyl-\(\beta\)-nitropropionate and \(\beta\)-nitropropionic acid are fairly soluble in chloroform, whereas the endecaphyllins are only very slightly soluble if at all. They are, however, quite soluble in ethanol. One can therefore assume that the weak nitro band observed in the chloroform extract stems mostly from the ethyl-\(\beta\)-nitropropionate and the acid. The strong nitro band in the ethanol extract, however, must be due to the endecaphyllins. This leads to the assumption that the true ratio of endecaphyllins to \(\beta\)-nitropropionic acid and its ethyl ester in the plant is larger than 2:1 as indicated by the data in Table I.
<table>
<thead>
<tr>
<th>Compounds and Yields Isolated from <em>I. endecaphylla.</em></th>
<th>Compound</th>
<th>m.p.</th>
<th>Formula</th>
<th>% yield(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl-(\beta)-nitropropionate</td>
<td>b.p. 67°/1 mm.</td>
<td>C(_5)H(_9)O(_4)N</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>(\beta)-Nitropropionic acid</td>
<td>66.5-67.5°</td>
<td>C(_3)H(_5)O(_4)N</td>
<td>5.56</td>
<td></td>
</tr>
<tr>
<td>Endecaphyllin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>139-140°</td>
<td>C(<em>{16})H(</em>{26})O(_{12})N(_2)</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>120-122°</td>
<td>C(<em>{15})H(</em>{21})O(_{15})N(_3)</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>A(_1)</td>
<td>81-91°</td>
<td>C(<em>{15})H(</em>{21})O(_{15})N(_3)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>A(_2)</td>
<td>156-158.5°</td>
<td>C(<em>{15})H(</em>{21})O(_{15})N(_3)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Succinic acid</td>
<td>184-186°</td>
<td>C(_4)H(_6)O(_4)</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>125-126.5°</td>
<td>C(<em>{15})H(</em>{21})O(_{15})N(_3)</td>
<td>2.13</td>
<td></td>
</tr>
<tr>
<td>B(_1)</td>
<td>129-130°</td>
<td>C(<em>{15})H(</em>{21})O(_{15})N(_3)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>155-156°</td>
<td>C(<em>{15})H(</em>{21})O(_{15})N(_3)</td>
<td>4.70</td>
<td></td>
</tr>
<tr>
<td>C(_1)</td>
<td>145-146.5°</td>
<td>C(<em>{12})H(</em>{18})O(_{12})N(_2)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>145-146°</td>
<td>C(<em>{12})H(</em>{18})O(_{12})N(_2)</td>
<td>2.22</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>132-134°</td>
<td>C(<em>{12})H(</em>{18})O(_{12})N(_2)</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>X((a))</td>
<td>104-105.5°</td>
<td>C(<em>{18})H(</em>{24})O(_{18})N(_4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\beta)-Methyl-D-glucoside</td>
<td>112-114°</td>
<td>C(<em>7)H(</em>{14})O(_6)</td>
<td>1.11</td>
<td></td>
</tr>
</tbody>
</table>

(a) Crude endecaphyllin X was isolated and supplied by Dr. M.P. Morris. This compound, however, could not be isolated in the present work.

(b) The yield represents the percentage of purified compound obtained, based on the amount of solvent free extract chromatographed. Multiplication by a factor of 10\(^{-2}\) gives the percent yield based on dry plant material.
Identification and Characterization.

**Ethyl-β-nitropropionate.**—Early in the chromatography a yellow liquid was isolated, the first material which showed the spectral characteristics of a nitro compound ($\nu_{\text{film}}^{36(a)} = 1555 \text{ cm}^{-1}; \lambda_{\text{max}}^{95\%} \text{ ethanol} = 270 \text{ m}\mu$). Furthermore, an infrared absorption band at 1745 cm$^{-1}$ indicated a saturated ester grouping. Distillation afforded a colorless liquid, b.p. 40-50° at 0.04 mm., which gave microanalytical data consistent with its formulation as $C_7H_9O_4N$. In view of the relatively low boiling point of the compound it is likely that the yield of 1.06% (Table I) is too low, since the solvent was removed from the chromatographic fractions at about 80°/10 mm. From the combined spectral and microanalytical data and the reported occurrence of β-nitropropionic acid in *I. endecaphylla* it was assumed that the isolated liquid was most likely ethyl-β-nitropropionate. The n.m.r. spectrum left no doubt about the identity of the compound. All the hydrogens were unambiguously defined. The ethyl group showed a triplet ($8.73 \tau; J = 7.3 \text{ c.p.s.}$) for the methyl hydrogens and a quartet ($5.78 \tau; J = 7.3 \text{ c.p.s.}$) for its methylene hydrogens. A triplet at $7.03 \tau, J = 5.8 \text{ c.p.s.}$, was assigned to the $\alpha$-hydrogens and another triplet at $5.31 \tau, J = 5.8 \text{ c.p.s.}$, to the $\beta$-hydrogens.
Its actual identification was accomplished by comparison of its infrared and ultraviolet spectra, as well as its gas chromatographic retention time (113°, 75 ml. per min., 20% SF-96 silicone on fire brick), with those of a synthetic sample of ethyl-β-nitropropionate. The ester was obtained in 78% yield by acid catalized esterification of the acid with ethanol.

Naturally occurring acids are frequently found as esters, in particular the corresponding methyl esters. However, the natural occurrence of ethyl esters is very rare. Therefore the possibility that ethyl-β-nitropropionate was formed on the column during the elution of β-nitropropiolic acid was considered. It is, however, unlikely because no ethanol was used at this early stage of the chromatography where ethyl-β-nitropropionate was isolated. Furthermore, the possibility that formation of the ester took place on the column from ethanol which may have been present in the extract was rejected when an ethanol solution of β-nitropropiolic acid (3%) and a suspension of silica gel (30%), stirred for 12 days at room temperature, yielded only 4% of the ester.

β-Nitropropiolic Acid. - The first crystalline compound obtained on chromatography, m.p. 66.5-67.5°, is water soluble and strongly acidic. Its infrared spectrum
(KBr) exhibits a typical broad acid hydroxyl absorption at 3000 cm\(^{-1}\), and a carbonyl and nitro band at 1715 and 1560 cm\(^{-1}\) respectively. In the ultraviolet region the nitro group absorbs at \(\lambda_{max}^{95\%}\) ethanol 273 \(\mu\). The melting point, microanalytical and spectral data left little doubt that this compound was the expected \(\beta\)-nitropropionic acid, especially since it was already earlier isolated\(^{15}\) from \textit{I. endacaphylla}. However, for additional confirmation \(\beta\)-nitropropionic acid was synthesized\(^{39}\) from \(\beta\)-propiolactone and sodium nitrite. The synthetic sample gave identical infrared and ultraviolet spectra with those of the naturally occurring acid, and the melting point (66.5-67.5\(^o\)) showed no depression on admixture of the acid isolated from the plant.

The n.m.r. spectrum shows two triplets for the \(\alpha\) and \(\beta\)-hydrogens at 6.92 \(\tau\) (\(J=5.8\) c.p.s.) and 5.31 \(\tau\) (\(J=5.8\) c.p.s.) respectively. The hydroxyl hydrogen appears at -1.19\(\tau\). The \(\alpha\)-hydrogens in the acid are shifted downfield by about 0.1\(\tau\) in comparison to the ethyl ester.

**Succinic Acid.** A second acidic crystalline compound, m.p. 184-186\(^o\), was obtained during the course of chromatography (Table I). Its infrared spectrum (KBr) exhibits the broad acid hydroxyl band at 3100 cm\(^{-1}\), and a carbonyl band at 1715 cm\(^{-1}\). From this spectrum,
the microanalytical data, and the neutral equivalent it was concluded that the compound is most likely succinic acid. This was confirmed by a mixed melting point with an authentic sample which showed no depression and by comparison of their infrared spectra which are indistinguishable.

The natural occurrence of this acid is not unusual since succinic acid has been found to be present in about 30 plant families.\textsuperscript{38(b)}

\textit{\&-Methyl-D-Glucoside.\textemdash} The last crystalline compound obtained in the chromatography existed in two dimorphic forms, m.p. 92-94\textdegree, and m.p. 112-114\textdegree. Its infrared spectrum (KBr) shows a strong hydroxyl band at 3430 cm\textsuperscript{-1}, the only band in the spectrum which is attributable to a functional group. This spectrum, combined with the microanalytical data, a positive Molisch test, and the high water solubility of the compound indicated a carbohydrate. The microanalysis revealed one O-methyl group (Zeisel) in the molecule. However, the microanalytical data did not distinguish between an O-methyl hexose or an O-methyl pentose. The same was true for the completely acylated and methylated derivatives as shown in Table II. Only with the preparation of a benzylidene derivative did its microanalytical data become decisive for an O-methyl hexose.
Bollenbach's book on methylglucosides furnishes melting points and optical rotation of β-methyl-D-glucoside in good agreement with the findings on the natural product. Additional comparison of the melting points of the prepared derivatives with those reported left little doubt that the isolated compound is β-methyl-D-glucoside. The actual identification as β-methyl-D-glucoside was then verified by mixed melting point with an authentic sample, and infrared spectra, and X-ray powder diffraction pattern comparison. Furthermore, its n.m.r. spectrum shows a doublet at 5.76 τ, J=7.4 c.p.s. which was assigned by Van Der Veen to the hydrogen on C₁ (lit. 5.62 τ, J=7.4 c.p.s.) and a singlet at 6.61 τ for the methoxyl hydrogens. The spectrum was taken in a 15% D₂O solution with dioxane as internal standard (6.43).

Although β-methyl-D-glucoside is a well known commercially available compound its natural occurrence is not too common. Previous isolation was reported in 9 species of the family of Dipsaceae, and Ceanothus azurens Hort.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Microanalysis</th>
<th>C</th>
<th>H</th>
<th>O</th>
<th>CH₃O⁻</th>
<th>CH₃CO⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent isolated</td>
<td></td>
<td>Found</td>
<td>41.67</td>
<td>7.44</td>
<td>50.72</td>
<td>15.60</td>
<td></td>
</tr>
<tr>
<td>O-Methyl pentose</td>
<td>C₆H₁₂O₅ 1/2 H₂O</td>
<td>Calcd.</td>
<td>41.61</td>
<td>7.57</td>
<td>50.82</td>
<td>17.90</td>
<td></td>
</tr>
<tr>
<td>O-Methyl hexose</td>
<td>C₇H₁₄O₆ 1/2 H₂O</td>
<td>Calcd.</td>
<td>41.41</td>
<td>7.45</td>
<td>51.23</td>
<td>15.26</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td></td>
<td>Found</td>
<td>49.56</td>
<td>6.04</td>
<td>44.14</td>
<td></td>
<td>44.64</td>
</tr>
<tr>
<td>O-Methyl-triacetyl pentose</td>
<td>C₁₂H₁₈O₈</td>
<td>Calcd.</td>
<td>49.65</td>
<td>6.25</td>
<td>44.10</td>
<td></td>
<td>44.4</td>
</tr>
<tr>
<td>O-Methyl-tetraacetyl hexose</td>
<td>C₁₅H₂₂O₁₀</td>
<td>Calcd.</td>
<td>49.72</td>
<td>6.12</td>
<td>44.16</td>
<td></td>
<td>47.4</td>
</tr>
<tr>
<td>Methylether</td>
<td></td>
<td>Found</td>
<td>52.54</td>
<td>8.80</td>
<td>38.99</td>
<td>59.7</td>
<td></td>
</tr>
<tr>
<td>Tetra-O-methyl pentose</td>
<td>C₉H₁₈O₅</td>
<td>Calcd.</td>
<td>52.41</td>
<td>8.80</td>
<td>38.79</td>
<td>60.1</td>
<td></td>
</tr>
<tr>
<td>Penta-O-methyl hexose</td>
<td>C₁₁H₂₂O₆</td>
<td>Calcd.</td>
<td>52.78</td>
<td>8.86</td>
<td>38.36</td>
<td>62.0</td>
<td></td>
</tr>
<tr>
<td>Benzyldiene</td>
<td></td>
<td>Found</td>
<td>59.31</td>
<td>6.58</td>
<td>33.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-Methyl-O-benzyldene pentose</td>
<td>C₁₃H₁₆O₅</td>
<td>Calcd.</td>
<td>61.89</td>
<td>6.39</td>
<td>31.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-Methyl-O-benzyldene hexose</td>
<td>C₁₄H₁₈O₆</td>
<td>Calcd.</td>
<td>59.56</td>
<td>6.43</td>
<td>34.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Endecaphyllins.

**General Properties.**—The endecaphyllins possess similar spectral, physical, and chemical properties and are therefore discussed as a group. They are white crystalline compounds which are soluble in polar solvents like acetone, tetrahydrofuran, or acetonitrile. From acetone-chloroform mixtures the compounds crystallize readily at room temperature. Some of the endecaphyllins have melting points (Table I) which are only a few degrees apart, or in one case even identical. However, their infrared spectra are different and mixed melting points depressed.

The infrared spectra (KBr) of all the endecaphyllins show bands at around 3500, 1740, and 1550 cm\(^{-1}\) which are either singlets, split, or have a shoulder. Furthermore, all the compounds have bands at around 1180 and 870 cm\(^{-1}\) in common. In general the spectra exhibit similar features with only small deviations. The absorption at 3500 cm\(^{-1}\) was attributed to OH rather than NH because the endecaphyllins are not basic, and no amide carbonyl band\(^{36(b)}\) was observed. The possibility of an imino group (-C=NH) was excluded since the spectra show no absorption for the C=N double bond (1640-1680).\(^{36(c)}\) From the bands at 1740 and 1180 cm\(^{-1}\) the
presence of ester groupings\textsuperscript{36(d)} was deduced. Bellamy\textsuperscript{36(a)} assigns absorptions in the 1550 cm\textsuperscript{-1} region to nitro groups which is in accord with the observations on ethyl-\(\beta\)-nitropropionate and \(\beta\)-nitropropionic acid.

The small amounts available and the insolubility in suitable solvents made the application of n.m.r. spectroscopy impossible. Furthermore, no mass spectroscopic data were obtained because of the non-volatility of the samples.\textsuperscript{43}

In the ultraviolet region the endecaphyllins show absorption maxima near 270 m\(\mu\) (Table III). Absorption of aliphatic nitro groupings in this region with extinction coefficients of the order of 25 \(\pm\) 13 have been reported.\textsuperscript{37} From the spectral properties the presence of nitro groups in the endecaphyllins was assumed. This assumption received further confirmation by a positive "red" reaction,\textsuperscript{44} indicating the formation of nitrolic acid from the \(-\text{CH}_2\text{NO}_2\) groupings present. The test was carried out on 1 mg. amounts of the endecaphyllins according to Dannley's\textsuperscript{44(b)} analytical method. With known primary and secondary nitro compounds a red-brown or green color reaction respectively was observed. Mixtures of primary and secondary nitro compounds showed colors which were distinguishable from that of either primary or secondary nitro groupings.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>μν</th>
<th>ε</th>
<th>ε/NO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl-β-nitropropionate</td>
<td>C₅H₉O₄N</td>
<td>270</td>
<td>21.2</td>
<td>21.2</td>
</tr>
<tr>
<td>β-Nitropropionic acid</td>
<td>C₃H₅O₄N</td>
<td>273</td>
<td>23.7</td>
<td>23.7</td>
</tr>
<tr>
<td>I</td>
<td>C₁₆H₂₆O₁₂N₂</td>
<td>276</td>
<td>39.8</td>
<td>19.9</td>
</tr>
<tr>
<td>A</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>272</td>
<td>73.0</td>
<td>24.3</td>
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<tr>
<td>A₁</td>
<td>C₁₅H₂₁O₁₅N₃</td>
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<td>74.6</td>
<td>24.9</td>
</tr>
<tr>
<td>A₂</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>270</td>
<td>106.8</td>
<td>34.3</td>
</tr>
<tr>
<td>B₁</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>275</td>
<td>75.5</td>
<td>25.2</td>
</tr>
<tr>
<td>C</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>277</td>
<td>75.7</td>
<td>25.2</td>
</tr>
<tr>
<td>C₁</td>
<td>C₁₂H₁₈O₁₂N₂</td>
<td>274</td>
<td>56.5</td>
<td>28.2</td>
</tr>
<tr>
<td>D</td>
<td>C₁₂H₁₈O₁₂N₂</td>
<td>273</td>
<td>51.0</td>
<td>25.5</td>
</tr>
<tr>
<td>E</td>
<td>C₁₂H₁₈O₁₂N₂</td>
<td>276</td>
<td>37.0</td>
<td>18.5</td>
</tr>
<tr>
<td>X</td>
<td>C₁₈H₂₄O₁₈N₄</td>
<td>274</td>
<td>99.0</td>
<td>24.8</td>
</tr>
</tbody>
</table>

*A₂ was not obtained in sufficient yield.*
The microanalytical data (Table IV) indicated one to three nitrogen atoms per molecule. From the size of the extinction coefficients (Table III) for the ultraviolet absorption the conclusion was drawn that all the nitrogen atoms in the endecaphyllins are present in nitro groups. In addition, quantitative microhydrogenation experiments (Table V) provided hydrogen uptake values in good agreement with those expected for complete reduction of all nitro groups to amino groups, i.e.,

\[ R-\text{NO}_2 + 3 \text{H}_2 \rightarrow R-\text{NH}_2 + 2 \text{H}_2\text{O} \]

Initial attempts at the reduction with various catalysts such as Pd, Pt, and Ru on charcoal or PtO₂ in solvents like ethanol, acetic acid, or dimethylformamide at room temperature and atmospheric pressure showed the compounds to be either resistant to reduction or to absorb hydrogen at such a slow pace that the results were erratic. This was also the case with known compounds such as ethyl-β-nitropropionate and β-nitropropionic acid. Carter²⁶ has also remarked on the resistance of karakin to catalytic reduction. No improvement resulted from the addition of a trace of perchloric acid to the solution. Addition of a trace of sodium hydroxide
<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Microanalysis</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>C₁₆H₂₆O₁₂N₂</td>
<td>Found 43.93 5.68 43.44 6.05</td>
<td>386</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 43.87 6.00 43.84 6.40</td>
<td>438</td>
</tr>
<tr>
<td>A</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>Found 37.19 4.25 49.73 8.82</td>
<td>436</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 37.30 4.38 49.69 8.70</td>
<td>483</td>
</tr>
<tr>
<td>A₁</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>Found 37.39 4.72 49.47 8.62</td>
<td>379</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 37.30 4.38 49.69 8.70</td>
<td>483</td>
</tr>
<tr>
<td>A₂</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>Found 37.38 4.20 - 8.88</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 37.30 4.38 49.69 8.70</td>
<td>483</td>
</tr>
<tr>
<td>B</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>Found 37.36 4.56 49.26 8.53</td>
<td>458</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 37.30 4.38 49.69 8.70</td>
<td>483</td>
</tr>
<tr>
<td>B₁</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>Found 37.68 4.43 48.62 8.49</td>
<td>473</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 37.30 4.38 49.69 8.70</td>
<td>483</td>
</tr>
<tr>
<td>C</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>Found 37.38 4.42 48.42 8.67</td>
<td>407</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 37.30 4.38 49.69 8.70</td>
<td>483</td>
</tr>
<tr>
<td>C₁</td>
<td>C₁₂H₁₈O₁₂N₂</td>
<td>Found 37.93 4.80 - 7.47</td>
<td>475</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 37.73 4.75 50.26 7.33</td>
<td>382</td>
</tr>
<tr>
<td>D</td>
<td>C₁₂H₁₈O₁₂N₂</td>
<td>Found 37.96 4.83 48.75 7.35</td>
<td>369</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 37.73 4.75 50.26 7.33</td>
<td>382</td>
</tr>
<tr>
<td>E</td>
<td>C₁₂H₁₈O₁₂N₂</td>
<td>Found 37.43 5.09 49.52 7.81</td>
<td>357</td>
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<tr>
<td></td>
<td></td>
<td>Calcd. 37.73 4.75 50.26 7.33</td>
<td>382</td>
</tr>
<tr>
<td>X</td>
<td>C₁₈H₂₄O₁₈N₄</td>
<td>Found 36.72 4.40 47.54 9.11</td>
<td>576</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcd. 36.99 4.14 49.28 9.59</td>
<td>584</td>
</tr>
</tbody>
</table>

The given data were deduced from duplicate analyses. Molecular weights were determined by the Rast method.
### TABLE V
**Hydrogenation of the Endecaphyllins.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>No. of NO₂-groupings</th>
<th>H₂-uptake in molar equivalents</th>
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</thead>
<tbody>
<tr>
<td>Ethyl-β-nitropropionate</td>
<td>C₅H₉O₄N</td>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td>β-Nitropropionic acid</td>
<td>C₃H₅O₄N</td>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td>I</td>
<td>C₁₆H₂₆O₁₂N₂</td>
<td>2</td>
<td>6.0</td>
</tr>
<tr>
<td>A</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>3</td>
<td>9.0</td>
</tr>
<tr>
<td>A₁</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>3</td>
<td>9.0</td>
</tr>
<tr>
<td>A₂</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>3</td>
<td>9.0</td>
</tr>
<tr>
<td>B</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>3</td>
<td>9.0</td>
</tr>
<tr>
<td>B₁</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>3</td>
<td>9.0</td>
</tr>
<tr>
<td>C</td>
<td>C₁₅H₂₁O₁₅N₃</td>
<td>3</td>
<td>9.0</td>
</tr>
<tr>
<td>C₁</td>
<td>C₁₂H₁₈O₁₅N₂</td>
<td>2</td>
<td>6.0</td>
</tr>
<tr>
<td>D</td>
<td>C₁₂H₁₈O₁₂N₂</td>
<td>2</td>
<td>6.0</td>
</tr>
<tr>
<td>E</td>
<td>C₁₂H₀O₁₂</td>
<td>2</td>
<td>6.0</td>
</tr>
<tr>
<td>X</td>
<td>C₁₈H₂₄O₁₈N₄</td>
<td>4</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Insufficient material was available in the cases where no values are recorded.*
accelerated the reduction markedly, but the hydrogen uptake was too low. However, reductions carried out in the presence of a trace of added triethyl amine (0.05% v/v), using Pt/C as catalyst and ethanol as solvent, furnished acceleration and reproducible results in good agreement with the calculated uptake. That the added amine so markedly accelerates the reduction may be an indication that the aci-nitro form of the substance is involved. The recently reported inhibition of the catalytic reduction (Pd/C) of aryl-nitro compounds by potassium- or sodium nitrite is not very likely in the present case, however, the presence of trace amounts of nitrite ions cannot be excluded.

Upon hydrolysis of the endecaphyllins in 1 N hydrochloric acid and subsequent paper chromatography, β-nitropropionic acid and glucose were detected, along with several unidentified compounds which probably result from partial hydrolysis. The fact that glucose and β-nitropropionic acid were recognized as hydrolysis products of the endecaphyllins, together with the infrared absorptions, suggested strongly that the endecaphyllins were β-nitropropionyl esters of glucose. Consideration of the combined spectral properties, micro-hydrogenation data, and the empirical formulae,
deduced from the microanalytical data (Table IV), led to the following classification.

The endecaphyllins C₁, D, and E are considered to be isomeric di-O-(β-nitropropionyl)-D-glucopyranoses, (17). The endecaphyllins A, A₁, A₂, B, B₁, and C are formulated as isomeric tris-O-(β-nitropropionyl)-D-glucopyranoses, (18) and endecaphyllin X as tetra-O-(β-nitropropionyl)-D-glucopyranose, (19).

\[
\text{C}_6\text{H}_{10}\text{O}_6(\text{COCH}_2\text{CH}_2\text{NO}_2)_2 \quad (17)
\]

\[
\text{C}_6\text{H}_9\text{O}_6(\text{COCH}_2\text{CH}_2\text{NO}_2)_3 \quad (18)
\]

\[
\text{C}_6\text{H}_8\text{O}_6(\text{COCH}_2\text{CH}_2\text{NO}_2)_4 \quad (19)
\]

Consideration of the large number of naturally occurring D-glucose derivatives makes it highly improbable that the endecaphyllins are derivatives of L-glucose. Applying the same arguments one is relatively safe in ruling out the corresponding furanose forms. Not enough material is available in order to check this hypothesis by hydrolizing the endecaphyllins, isolating the glucose residue, and measuring the optical rotation. An attempt
to isolate the glucose from the hydrolized crude extract by paper chromatography proved to be futile.

The assignment of di-, tri-, and tetra-esters of glucose for the endecaphyllins was also well supported by the individual saponification equivalents (Table VI). Twice as many equivalents of sodium hydroxide were used up as expected for the ester hydrolysis. Treatment of \( \beta \)-nitropropionic acid under the same conditions, however, showed that the aci-nitro form is involved which accounts for the second equivalent of sodium hydroxide, i.e.,

\[
O_2N-CH_2CH_2-O-OR + 2NaOH \rightarrow NaO-N=CH-CH_2O-ONa + ROH + 2H_2O
\]

The observed Rf values (0.5-0.6 for diesters, and 0.7-0.8 for triesters) of the pure endecaphyllins on silica gel thin layers are also in good accord with the assignment. They show the same sequence as noted for the order of elution on column chromatography (Table I).

Furthermore, the infrared spectra allow a qualitative correlation of the relative strength of the hydroxyl band versus the C-H band (around 3000 cm\(^{-1}\)) in good agreement with the proposal.
Table VI
Saponification Equivalents.

<table>
<thead>
<tr>
<th>Endecaphyllins</th>
<th>Formula</th>
<th>Molar equivalents of base used up.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a) Found</td>
<td>(b) Calcd.</td>
</tr>
<tr>
<td>I</td>
<td>$\text{C}<em>{16}\text{H}</em>{26}\text{O}_{12}\text{N}_2$</td>
<td>4.00</td>
</tr>
<tr>
<td>A</td>
<td>$\text{C}<em>{15}\text{H}</em>{21}\text{O}_{15}\text{N}_3$</td>
<td>6.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.00</td>
</tr>
<tr>
<td>$A_1$</td>
<td>$\text{C}<em>{15}\text{H}</em>{21}\text{O}_{15}\text{N}_3$</td>
<td>6.16</td>
</tr>
<tr>
<td>$A_2$</td>
<td>$\text{C}<em>{15}\text{H}</em>{21}\text{O}_{15}\text{N}_3$</td>
<td>6.00</td>
</tr>
<tr>
<td>B</td>
<td>$\text{C}<em>{15}\text{H}</em>{21}\text{O}_{15}\text{N}_3$</td>
<td>6.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.00</td>
</tr>
<tr>
<td>$B_1$</td>
<td>$\text{C}<em>{15}\text{H}</em>{21}\text{O}_{15}\text{N}_3$</td>
<td>5.96</td>
</tr>
<tr>
<td>$C_1$</td>
<td>$\text{C}<em>{12}\text{H}</em>{18}\text{O}_{12}\text{N}_2$</td>
<td>5.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.00</td>
</tr>
<tr>
<td>$C_1$</td>
<td>$\text{C}<em>{12}\text{H}</em>{18}\text{O}_{12}\text{N}_2$</td>
<td>3.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.00</td>
</tr>
<tr>
<td>$D$</td>
<td>$\text{C}<em>{12}\text{H}</em>{18}\text{O}_{12}\text{N}_2$</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.00</td>
</tr>
<tr>
<td>$E$</td>
<td>$\text{C}<em>{12}\text{H}</em>{18}\text{O}_{12}\text{N}_2$</td>
<td>4.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.00</td>
</tr>
<tr>
<td>$X$</td>
<td>$\text{C}<em>{18}\text{H}</em>{24}\text{O}_{12}\text{N}_4$</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.00</td>
</tr>
</tbody>
</table>

(a) Most of the values are deduced from duplicate saponifications. Endecaphyllin I and $A_2$ were not available in sufficient quantity.

(b) The values are calculated for one equivalent per ester grouping plus one equivalent per aco-nitro group as discussed above.
**Endecaphyllin A.-** This compound (m.p. 120-122°; 
\[\alpha\]_D^{22} = 4.3°) is probably identical with the reported karakin\textsuperscript{26} (m.p. 120°; \[\alpha\]_D^{20} = 4.5°), for which the structure 1,4,6-tris-O-(\beta-nitropropionyl)-D-glucopyranose was proposed (p.12). However, it was not possible to prepare the reported diacetate, m.p. 103°, but a monoacetate, m.p. 124.5-126.5°, was obtained instead. Repeated acid catalized acetylation with acetic anhydride afforded only the monoacetate. The acetylation, however, was carried out on a much smaller scale than the one reported in the case of karakin (5 g.). Base catalized acetylation (pyridine) led to resinous material which could not be characterized. The base sensitivity is discussed in a later section. Microhydrolysis of 1 mg. of endecaphyllin A in hot water yielded \(\beta\)-nitropropionic acid (identified by paper chromatography) and a few crystals, m.p. 101.5-104°, presumably a diester resulting from loss of one \(\beta\)-nitropropionyl group. On hydrolysis of a 55 mg. sample, 80% of the starting material was recovered, along with very little crystalline \(\beta\)-nitropropionic acid. In each case no glucose could be detected by paper chromatography. A similar hydrolysis of karakin\textsuperscript{26} (exact conditions are not reported), however, afforded on column chromatography of the hydrolysate: D-glucose, a
crystalline diester, m.p. 138°, and an oil regarded to be a mixture of isomeric monoesters.

**Endecaphyllin B₁**.- Endecaphyllin B₁ was originally considered to be a triester of β-methyl-D-glucoside based on its high carbon content (C, 38.54%). A duplicate analysis, however, furnished results (Table IV) satisfactory for a triester of glucose. In addition, upon basic hydrolysis of B₁ and thin layer chromatographic analysis glucose was identified. A ketal as present in the methyl-D-glucoside would resist base hydrolysis.

**Endecaphyllin I**.- This compound does not fit into the pattern of simple O-(β-nitropropionyl)-D-glucopyranoses. From its analysis an empirical formula of C₁₆H₂₆O₁₂N₂ was deduced. Its ultraviolet absorption (Table III) accords with the 2 nitrogens being present as nitro groupings. The infrared spectrum shows the usual features of the other endecaphyllins, however, exhibiting a better developed doublet in the carbonyl region (1745, 1710 cm⁻¹). This is also true for the nitro band (1575, 1553 cm⁻¹, doublet), which is, in addition, shifted slightly to higher frequency. From the combined spectral and analytical data it was tempting to assume a di-O-(β-nitropropionyl)-D-glucopyranose derivative containing a C₄H₉O-ether grouping,
$C_6H_{10}O_6(COCH_2-CH_2-NO_2)_2(C_4H_9)$. Base as well as acid hydrolysis, however, furnished glucose ruling out any $C_4H_9O$-ether grouping. With the limited data on hand one can only conclude that the additional 4 carbon atoms must be contained in the nitro ester groupings with the nitro groups on terminal positions (positive "red" reaction$^4$). Unfortunately, there is not enough material available in order to isolate and identify this hypothetical new nitro acid.
Attempted Structural Elucidation of Positional Isomers.

Exhaustive methylation is a classical method to locate hydroxyl groups in carbohydrate chemistry. In view of this, methylation of the hydroxyl groups present in the endecaphyllins and subsequent base hydrolysis of the $\beta$-nitropropionyl groupings leading to known $\alpha$-methyl-ethers of glucose or methyl glucoside appeared to be a promising route.

\[
\begin{align*}
C_6H_{12-n}O_6(COCH_2CH_2NO_2)_n & \xrightarrow{a} C_6H_{12-(n+m)}O_6(COCH_2CH_2NO_2)_n(CH_3)_m \\ 
C_6H_{12-n}O_6(CH_3)_m & \xrightarrow{b} \\

\end{align*}
\]

a) Methylation ; b) Hydrolysis.

Kuhn's method of base catalized methylation (barium oxide-barium hydroxide-methyl iodide) when applied to endecaphyllin C furnished tarry products. This extreme susceptibility of the compound towards base (even pyridine, p.37) suggested that methylation under acidic
conditions might be more fruitful. The procedure of Neeman, in which methylation of estriol-mono-gluco-
sidoronic acid$^{50}$ (20) with diazomethane and boron trifluoride as a catalyst led to the fully methylated com-
 pound 21 in approximately 50% yield, was appealing as an adoptable method for the endecaphyllins.

\[ \text{(20)} \]

\[ \text{(21)} \]

a) Diazomethane and ethanol as solvent.
b) Diazomethane-boron trifluoride etherate, methylene chloride as solvent.
Extensive studies of this general reaction\(^{51}\) had suggested that methylene chloride was the most suitable solvent, since ethers seem to tie up the catalyst. This presented a serious handicap because the endecaphyllins were insoluble in this solvent.

In order to check the stability of \(\beta\)-nitropropionyl glucosides during the reaction, a synthetic sample of tetra-\(O\)-(\(\beta\)-nitropropionyl)-\(\beta\)-methyl-\(D\)-glucoside (see next section) was submitted to the boron trifluoride catalized methylation conditions. A quantitative recovery of starting material was possible.

When treatment of a suspension of synthetic tris-\(O\)-(\(\beta\)-nitropropionyl)-\(\beta\)-methyl-\(\beta\)-\(D\)-glucoside, m.p. 147-148°, (see next section) in methylene chloride, with a 16 fold excess of diazomethane and catalytic amounts of boron trifluoride afforded a methyl ether derivative in good yield, the problem appeared near solution. The infrared spectrum of the crystalline compound (m.p. 126-127.5°) obtained, shows a weak hydroxyl absorption at 3525 cm\(^{-1}\). Its microanalytical data, however, were in good agreement with those expected for a tris-\(O\)-(\(\beta\)-nitropropionyl)-\(O\)-methyl-\(\beta\)-methyl-\(D\)-glucoside. In addition, base hydrolysis and chromatography of the hydrolysate on a silica gel thin layer showed a Rf value of 0.34 (glucose 0.18;
β-methyl-D-glucoside 0.28) which is well within the differentiating order of reported values for O-methyl ethers of glucosides$^5$. Application of this methylation procedure to a suspension of endecaphyllin C in methylene chloride gave very disappointing results. Repeated attempts with a 30-40 fold excess of diazomethane and the corresponding amounts of catalyst afforded only about 30% of a mixture of methylated material. This mixture separated on silica gel thin layers into three components of Rf 0.71; 0.75; 0.93 (endecaphyllin 0, 0.76). Remethylation of this mixture did not improve the distribution of products. It was also not possible to obtain any crystalline compounds or marked separation on column chromatography (silica gel B.D.H.).

Expecting better results in a homogeneous reaction mixture tetrahydrofuran was used as a solvent for endecaphyllin C. In order to counteract the possibly serious interaction of the solvent with the catalytic amounts of boron trifluoride, larger amounts of catalyst were employed. Only about 10% of starting material was recovered, but the methylated product was again a mixture. In addition, a weight increase of 30-40% was observed. Chromatography of this mixture showed 3 compounds
compounds (silica gel thin layer, Rf 0.75; 0.94; 1.0; on paper, Rf 0.87; 0.97). Doubling the amount of catalyst during the methylation afforded the same product mixture.

On several crystallizations of this mixture, about 25% of the total amount was obtained as a pure crystalline compound (m.p. 98-101°; Rf 0.75 on silica gel, or 0.87 on paper). From microanalytical data, and the saponification equivalent, the conclusion was made that the compound is a di-0-(β-nitropropionyl)-0-methyl-D-glucose (22). Acidic hydrolysis and thin layer chromatographic analysis (Rf 0.20; β-methyl-D-glucoside Rf 0.19) confirmed this assignment. Since the 0-methyl ether of glucose survived the acid hydrolysis, the methoxyl cannot be located at C1. It appears, however, very likely that the lost β-nitropropionyl grouping from endecaphyllin C was located at C1, because no loss of β-nitropropionyl-groupings was observed on subjecting tetra-0-(β-nitropropionyl)-β-methyl-D-glucoside to the same reaction conditions. The second compound (Rf 0.94) observed among the methylation products stems presumably from a higher degree of methylation of compound 22, which would be either a di-0-methyl-di-0-(β-nitropropionyl)-D-glucose (23) or tris-0-methyl-di-0-(β-nitropropionyl)-D-glucose (24). The component at Rf 1.0 appears to be a by-product
from tetrahydrofuran which caused the abnormal weight increase observed on work up of the reaction.

From the 3 components obtained on methylation in methylene chloride, the component at Rf 0.75 is presumably identical with the isolated di-0-(β-nitropropionyl)-0-methyl-D-glucopyranose (22), m.p. 98-101°; Rf 0.75, and the one at Rf 0.94 is identical with the second component observed on methylation in tetrahydrofuran. The new component at Rf 0.71 might well be the desired product of di-0-methyl-tris-0-(β-nitropropionyl)-D-glucose (25), since Rf 0.69 was observed for the 0-methyl-tris-(β-nitropropionyl)-β-methyl-D-glucoside (p.86). The discussed methylation of endecaphyllin C (26) is summarized in the following equations, i.e.,

\[
\begin{align*}
&\text{C}_6\text{H}_9\text{O}_6(\text{COCH}_2\text{CH}_2\text{NO}_2)_2(\text{Me}) \quad \text{(22)} \\
&\text{C}_6\text{H}_9\text{O}_6(\text{COCH}_2\text{CH}_2\text{NO}_2)_2(\text{Me})_2 \\
&\text{C}_6\text{H}_9\text{O}_6(\text{COCH}_2\text{CH}_2\text{NO}_2)_3 \quad \text{(26)} \\
&\text{C}_6\text{H}_7\text{O}_6(\text{COCH}_2\text{CH}_2\text{NO}_2)_2(\text{Me})_3 \quad \text{(24)} \\
&\text{C}_6\text{H}_7\text{O}_6(\text{COCH}_2\text{CH}_2\text{NO}_2)_3(\text{Me})_2 \quad \text{(25)}
\end{align*}
\]
One reason for the failure of the method might be the insolubility of endecaphyllin C in methylene chloride. This made it necessary to employ an unusually large excess of diazomethane and consequently more catalyst (also in THF) which possibly afforded the variety of products. Nevertheless, if larger amounts of material would be available, separation of the methylation products by column chromatography might lead to success.

An alternate route to known O-methyl-D-glucopyranosides using tetrahydropyranyl protecting groups for the free hydroxyls present in endecaphyllins was considered:

\[
\begin{align*}
\text{C}_6\text{H}_{12-n}^6\text{COC}_2\text{H}_4\text{NO}_2)^n + \text{DHP} & \xrightarrow{\text{H}^+} \text{C}_6\text{H}_{12-(n+m)}^6\text{COC}_2\text{H}_4\text{NO}_2)^n\text{C}_5\text{H}_{10}^0)^m \\
& \xrightarrow{\text{base}} \text{C}_6\text{H}_{12-(n+m)}^6(\text{Me})_n\text{C}_5\text{H}_{10}^0)^m \leftrightarrow \text{C}_6\text{H}_{12-m}^6\text{C}_5\text{H}_{10}^0)^m \\
& \xrightarrow{\text{acid}} \text{C}_6\text{H}_{12-n}^6(\text{Me})_n + \text{C}_6\text{H}_{12-(n+1)}^6(\text{Me})_{n-1}
\end{align*}
\]
Acid hydrolysis will remove either grouping at C₄ yielding type (27) in the case of a free hydroxyl at C₄ in the starting material or type (28) when C₄ was originally acylated.

For the success of this method it was essential to obtain high yields in the formation of the tetrahydropyranyl derivatives under very mild conditions (preferentially without acid), in order to prevent acyl migration on the endocaplyllins during the reaction.

Upon refluxing β-methyl-D-glucoside in tetrahydrofuran and dihydropyran, as reported in steroid chemistry only starting material was recovered. On reaction in tetrahydrofuran either at room temperature or at reflux, using a 20% excess of dihydropyran and a trace of p-toluene-sulfonic acid, mostly starting material was obtained, along with some partially substituted glucoside.

Applying a much larger excess (20-25 fold) of dihydropyran under the acid catalized conditions above, all the glucoside was transformed into a mixture of 6 tetrahydropyranyl derivatives (thin layer chromatographic analysis), and about 30-40% of a by-product derived from dihydropyran was observed.

At this point the method did not appear too promising and was therefore not further investigated.
Finally an equally logical route to known O-methyl ethers of glucose was considered. The reaction of the hydroxyl groups present in the endecaphyllins with phenylisocyanate would lead to the corresponding N-phenyl-carbamates. This protecting group allows acid hydrolysis of the β-nitropropionyl groups followed by methylation of the generated hydroxyl groups. Basic removal of the carbamate groupings would then result in known O-methyl ethers. However, reported low yields (25-30%) in the formation of N-phenylocarbamates54,55 along with the extreme ease of carbamyl migration (O₂ → O₆ about 90%; O₂ → O₄ about 15%) observed on acid hydrolysis of O-acetyl-O-carbamate-D-glucopyranose derivatives56 led to the rejection of this method.
Synthesis of Some O-(β-Nitropropionyl)-D-Glucopyranosides.

In view of the disappointing results obtained on methylation of the endecaphyllin C, and the small amounts isolated, the possibility of synthesizing one of these compounds in order to prove their structures became attractive. Particularly the possibility of synthesizing a triester was pertinent, since 6 out of 10 possible positional isomers (excluding the anomeric forms at O₁) of tris-O-(β-nitropropionyl)-D-glucopyranoses were found among the endecaphyllins. Furthermore, it is desirable to obtain a variety of O-(β-nitropropionyl)-D-glucoses in larger amounts, in order to investigate a possible relationship of their pharmacological activity with the interesting effects observed from I. endecaphylla Jacq.

In general, the acylation of glucose does not present any problems. However, it proved to be quite laborious to find conditions for the formation of O-β-nitropropionyl derivatives. Several base catalized acylation attempts using pyridine or collidine and β-nitropropionyl chloride proved futile. Staab’s imidazole method⁵⁷, as well as the carbodiimide method⁵⁸ (better known in peptide synthesis), which are also subject to base catalysis, supplied no better results.

The failure of base catalized acylation and the
observed extreme susceptibility to base of endecaphyllin A and C (p.37 and 40) might have its origin in the abstraction of the highly activated β-hydrogen of the β-nitropropionyl group, which in turn can cause a number of side reactions. Furthermore, the possibility of the elimination of the nitro grouping and polymerization reactions of the resulting acrylate has to be considered.

All efforts aimed at acid catalyzed acylation with β-nitropropionic anhydride failed. However, proton accepting solvents like tetrahydrofuran or N-methylpyrrolidone were used. An attempt to react glucose in a mixture of molten β-nitropropionic acid and its anhydride (HClO₄ as catalyst) also failed.

Finally acetylation of dextrose (29) with β-nitropropionyl chloride (1 mmole scale) in N-methylpyrrolidone as a solvent (no base present) afforded 9% of a tetra-O-(β-nitropropionyl)-D-glucopyranose derivative (30), m.p. 145-146.5°. Its infrared spectrum shows all the features exhibited by the endecaphyllins and is, in particular, very similar to that of endecaphyllin C.

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow \text{C}_6\text{H}_{6}\text{O}_6 (\text{COCH}_2\text{CH}_2\text{NO}_2)_4
\]

(29) (30)
Application of similar reaction conditions to \( \beta \)-methyl-\( D \)-glucoside (31) on a 10 mmole scale furnished the fully acylated product, tetra-\( 0\)-\(( \beta \)-nitropropionyl\)-\( \beta \)-methyl-\( D \)-glucoside (32), m.p. 109-110°, in 18.7% yield. In addition, 5% of a tris-\( 0\)-\(( \beta \)-nitropropionyl\)-\( \beta \)-methyl-\( D \)-glucoside (33), m.p. 144-146.5°, and about 20% of an oily mixture of partially acylated \( \beta \)-methyl-\( D \)-glucoside derivatives was obtained.

\[
\begin{align*}
\text{C}_7\text{H}_{10}\text{O}_6 \text{ (COCH}_2\text{CH}_2\text{NO}_2)_4} \\
\text{C}_7\text{H}_{14}\text{O}_6 & \rightarrow \text{C}_7\text{H}_{11}\text{O}_6 \text{ (COCH}_2\text{CH}_2\text{NO}_2)_3} \\
(32) & \\
(31) & \\
(33)
\end{align*}
\]

\( N \)-Methylpyrrolidone, in this acylation seems to be an effective proton acceptor, yet not sufficiently basic to cause serious side-reaction.

The \( \beta \)-nitropropionic acid was prepared according to Gresham's method\textsuperscript{39}. It was found, that the yields become more reproducible, when the aqueous reaction mixture is
buffered with one equivalent of ammonium chloride. Its ethyl ester (b.p. 45.5°/0.07 mm.) is formed in about 80% yield by acid catalized esterification. With thionyl chloride the corresponding acid chloride can be obtained in better than 90% yield (b.p. 67°/0.2mm.).

The β-nitropropionic anhydride (m.p. 64-65°) was prepared in 75% yield from the acid with methoxyacetylene. Amide formation from the acid chloride with piperidine occurred in only 35% yield (b.p. 120-140°/0.25 mm.).

The acid chloride and the N-piperidino amide have not been reported previously.
EXPERIMENTS

The infrared spectra were taken on the Perkin-Elmer Models number 137B, 21 and 237 spectrophotometers. The Cary Model 14 and the Perkin-Elmer 202 were used to take ultraviolet spectra. The n.m.r. spectra were run on the Varian Associates NMR Spectrophotometer operated at 60 mc. All chemical shifts are measured in tau values against tetramethylsilane as internal reference standard ($\tau = 10.00$), unless otherwise noted, and the coupling constants are given in c.p.s. Analyses were performed by the Mikroanalytisches Laboratorium im Max-Plank-Institut fuer Kohlenforschung. Unless otherwise noted, the melting points were taken on a Fischer-Johns melting point block and are uncorrected. The boiling points are also uncorrected.

The chloroform used in the isolation procedure was technical grade, distilled, and subsequently washed with water. All the other solvents employed in this work were also distilled. The silica gel used in the column chromatography was from the British Drug Houses (B.D.H.).
Microchromatographic Methods.

The crude extract was spotted on paper (Whatman No.3) and horizontally chromatographed with isopropanol - 5% aqueous ammonia 4:1. Development of the chromatogram with Kedde reagent (1% 3,5-dinitrobenzoic acid and 0.5% potassium hydroxide in methanol - water 1:1) showed nine poorly resolved major spots. Increase as well as decrease of the ammonia content in the solvent system diminished the resolution. This system was also used on the individual fractions of the initial column chromatography attempts. It helped to determine the degree of separation on silica gel (B.D.H.), aluminum oxide (Merk, acid washed), and cellulose powder (Whatman, standard grade). Micro-chromatographic methods on the crude extract as well as on the isolated endecaphyllins resulted either in streaking of the spots or very small differences in Rf values.

The solvent systems listed in Table VII have been tried on paper (Whatman No.3), and thin layers of aluminum oxide G (Merk) or silica gel G (Merk) without significant improvement. The thin layer plates were sprayed with 10 N sulfuric acid and developed at 120°. The paper chromatograms were developed with 0.1 N silver nitrate in water-methanol, dried, and subsequently sprayed with 2.5 N potassium hydroxide in ethanol-water.
**TABLE VII**

**Solvent Systems Used in Microchromatographic Attempts.**

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>v/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Amyl alcohol-t-amyl alcohol</td>
<td>1:1</td>
</tr>
<tr>
<td>2-Propanol-1.0% aq. ammonia</td>
<td>4:1</td>
</tr>
<tr>
<td>2-Propanol-0.5% aq. ammonia</td>
<td>4:1</td>
</tr>
<tr>
<td>2-Propanol-1.5% aq. ammonia</td>
<td>4:1</td>
</tr>
<tr>
<td>2-Propanol-7.0% aq. ammonia</td>
<td>4:1</td>
</tr>
<tr>
<td>Chloroform-methanol-water</td>
<td>10:4:10</td>
</tr>
<tr>
<td>Water sat'd, chloroform</td>
<td></td>
</tr>
<tr>
<td>Chloroform-acetone</td>
<td>95:5</td>
</tr>
<tr>
<td>Chloroform-ethanol</td>
<td>99:1</td>
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<td>Acetone-water</td>
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<tr>
<td>Acetone-water</td>
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<td>2-Butanone</td>
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<td>Methyl butyl ketone-water sat'd, chloroform</td>
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<td>Methyl butyl ketone-water sat'd, chloroform</td>
<td>2:8</td>
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<td>Methyl butyl ketone-water sat'd, chloroform</td>
<td>3:7</td>
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<tr>
<td>Phenol-water</td>
<td>88:12</td>
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<td>Phenol sat'd with 2M acetic acid</td>
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</tr>
<tr>
<td>Phenol-glacial acetic acid</td>
<td>95:5</td>
</tr>
<tr>
<td>Phenol (88% + 12% water)-chloroform-acetone</td>
<td>1:3:2</td>
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<td>Benzene-methanol</td>
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<tr>
<td>1-Butanol-acetic acid-water</td>
<td>4:1:1</td>
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<td>Pyridine-ethyl acetate-water</td>
<td>4.65:11.25:2.5</td>
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<tr>
<td>Benzene-ethyl acetate-water-acetic acid</td>
<td>200:47:15:1</td>
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<td>1-Butanol-acetic acid-ether-water</td>
<td>9:6:3:1</td>
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Separation attempts by electrophoresis failed completely. The following systems were used at a voltage of 1200-1400 V (D.C.), and a current of 50-70 mAmp.

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<tr>
<td>4.0</td>
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<tr>
<td>2.2</td>
<td>potassium biphthalate/hydrochloric acid</td>
</tr>
<tr>
<td>6.05</td>
<td>pyridine/formic acid</td>
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<tr>
<td>8.82</td>
<td>ammonia/acetic acid/water</td>
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<tr>
<td>10.0</td>
<td>sodium hydroxide/glycocol</td>
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</tbody>
</table>
Isolation.

A dark-brown acetone extract (about 400 g. of solvent-free material obtained from 40 kg. of dry plant material) of the leaves and stems of *I. endecaphylla* was supplied by Dr. M.P. Morris. From this extract 5.4 g. of solvent-free material was chromatographed on 650 g. of silica gel (B.D.H.), packed in Skelly Solve B. The column used was 700 mm. long and 45 mm. in diameter. With a flow rate of 100 ml./hr. 500 ml. cuts were collected. Skelly Solve B was slowly replaced by water saturated chloroform.

**β-Nitropropionic Acid.** Upon eluting with pure water saturated chloroform, the first crystalline compound was obtained (cut No. 27-41). The crude yellow material, m.p. 62.5-66°, was recrystallized 3 times from chloroform affording 300 mg. (5.56% yield) of white plates, m.p. 66.5-67.5°; υ (KBr) 3100, 1715, 1560 cm\(^{-1}\); λ \(95\%\) ethanol max 273 μ, e 23.7. The n.m.r. spectrum was taken in deuterochloroform (about 10% concentration). It showed a triplet at 6.92 τ (\(J=5.8\) c.p.s.) and another one at 5.31 τ (\(J=5.8\) c.p.s.), and a singlet at -1.19 τ.

**Anal.** Calcd. for C\(_3\)H\(_5\)O\(_4\)N: C, 30.26; H, 4.37; O, 53.75; N, 11.76; Mol. Wt., 119. Found: C, 30.52; H, 4.37; O, 52.07; N, 12.00; Mol. Wt., 132.
The mixed melting point with an authentic sample of
$\beta$-nitropropionic acid was undepressed ($66.5-67.5^\circ$). The
infrared spectra of the two samples were indistinguishable.

Continuation of the chromatography with unusually
small increases of ethanol (1% portions) to water satu­
rated chloroform furnished the endecaphyllins.

Endecaphyllin A.- With 2% ethanol in water saturated
chloroform (cuts No.76-84) tan crystals, melting at 109-
117$^\circ$, were obtained. Four recrystallizations from ace­
tone-chloroform gave 77 mg. (1.42% yield) of white crystals,
m.p. 120-122$^\circ$; $\nu$ $\text{KBr}$ max 3510, 1750, 1725 (shoulder), 1555
(split), 1372, 1170, 1093, 868 cm$^{-1}$; $\lambda$ 95% ethanol
$\text{max}$ 272 mp, $\epsilon$ 73.4; $[\alpha]_D^{22} = 4.3^\circ$ (5% in acetone; 1 dm.).

Anal. Calcd. for C$_{15}$H$_{21}$O$_{15}$N$_3$: C, 37.30; H, 4.38;
O, 49.69; N, 8.70; Mol. Wt., 483. Found: C, 37.19,
36.98; H, 4.20; 4.25; 0, 49.73, 48.82; N, 8.82, 8.87;
Mol. Wt., 436.

Succinic acid. - A semisolid fraction (cut No. 93-
105) was eluted with 3% ethanol in water saturated chloro­
form. When crystallization attempts failed, the mixture
was rechromatographed on silica gel (B.D.H.), which
yielded crystalline succinic acid with 3% ethanol in
water saturated chloroform as eluent. The crude material
melting at 150-164$^\circ$ was recrystallized 3 times from
acetone-hexane which afforded 16 mg. (0.3% yield) of white crystals, m.p. 184-186° (sealed tube, sublimes); ν\text{max} 3100 (broad), 1755 (shoulder), 1715 cm\(^{-1}\).

\textbf{Anal.} Calcd. for C\(_4\)H\(_6\)O\(_4\): C, 40.71; H, 5.13; O, 54.24, neut. equiv., 59. Found: C, 40.68; H, 5.15; O, 53.03; neut. equiv., 56.8.

Admixture with an authentic sample of succinic acid showed no depression of the melting point, (183-185° sealed tube, sublimes). The infrared spectra of the isolated material was indistinguishable from that of the authentic sample.

\textbf{Endecaphyllin A\(_1\)}.—All the oily fractions obtained from the rechromatography described above for the isolation of succinic acid were combined, and dissolved in an acetone-chloroform mixture. Storage in the refrigerator afforded after several months a small amount of white crystals, endecaphyllin A\(_1\). The filtered crude material, melting at 73-85°, was recrystallized 5 times from acetone-chloroform, which raised the melting range to 81-91°. Further recrystallizations did not result in sharp melting material. The 35 mg. (0.65% yield) of white crystals appeared to be uniform (Rf 0.71) when chromatographed on a thin layer of silica gel (Woelm, no binder) with 1-butanol-glacial acetic acid-water (4:1:1) as developing solvent system. The material showed
\[ \nu_{\text{KBr}} 3340 \text{ (split), 1745, 1555, 1370, 1177, 1035}, \]
\[ \text{max} \]
\[ 868 \text{ cm}^{-1}; \lambda_{\text{max}} \text{ ethanol} 273 \text{ m}\mu, \epsilon 74.5. \]
\[ \text{Anal. Calcd. for C}_{15}\text{H}_{21}\text{O}_{15}\text{N}_{3}: \text{ C}, 37.30; \text{ H}, 4.38; \]
\[ 0, 49.69; \text{ N}, 8.70; \text{ Mol. Wt.}, 483. \text{ Found: C}, 37.39; \text{ H}, 4.72; \]
\[ 0, 49.47; \text{ N}, 8.62, 8.26; \text{ Mol. Wt.}, 379, 357. \]

**Endecaphyllin B.** - Endecaphyllin B was obtained with the same solvent mixture of 3% ethanol in water saturated chloroform (cuts No.106-111). The isolated tan material, m.p. 112-119°, was recrystallized from acetone-chloroform, then 2 times from ethanol yielding 115 mg. (2.13% yield) of white needles melting at 125-126.5°;

\[ \nu_{\text{KBr}} 3530, 1765, \text{ (shoulder), 1738, 1555, 1400, 1255, 1179}, \]
\[ 1087, 872 \text{ cm}^{-1}; \lambda_{\text{max}} \text{ 95% ethanol} 270 \text{ m}\mu, \epsilon 106. \]
\[ \text{Anal. Calcd. for C}_{15}\text{H}_{21}\text{O}_{15}\text{N}_{3}: \text{ C}, 37.30; \text{ H}, 4.38; \]
\[ 0, 48.69; \text{ N}, 8.70; \text{ Mol. Wt.}, 483. \text{ Found: C}, 37.36, 37.47; \]
\[ \text{H}, 4.72, 4.56; 0, 49.26, 49.01; \text{ N}, 8.53, 8.47; \text{ Mol. Wt.}, \]
\[ 458, 352. \]

**Endecaphyllin C.** - Water saturated chloroform with 3.0-4.5% ethanol furnished endecaphyllin C as tan crystals (cuts No. 117-137). The isolated crude material melted at 135-148° (decomp.). Five recrystallizations from acetone-chloroform afforded 254 mg. (4.7% yield) of a white crystalline compound, m.p. 155-156° (very little decomp.);

\[ \nu_{\text{KBr}} 3600, 1745, 1551, 1385, 1200, 1025, 875 \text{ cm}^{-1}; \]
95% ethanol

\[ \lambda_{\text{max}} \] 270 \text{ m} \mu, \epsilon 75.7.

*Anal.* Calcd. for C\(_{15}\)H\(_{21}\)O\(_{15}\)N\(_3\): C, 37.30; H, 4.38; 0, 49.69; N, 8.70; Mol. Wt., 483. Found: C, 37.38, 37.39; H, 4.50, 4.42; 0, 48.42, 47.42; N, 8.67, 8.52; Mol. Wt., 407, 394.

**Endecaphyllin D.** - With 6.5% ethanol in water, saturated chloroform endecaphyllin D was eluted (cuts No.160-173). The crude material was tan crystals of m.p. 134-141\(^\circ\). Two recrystallizations from acetone-chloroform afforded 120 mg. (2.22% Yield) of white crystals, m.p. 145-146\(^\circ\); \(\nu \text{ KBr} \) 3500, 1733 (split), 1552, 1390, 1195, 1030, 873 cm\(^{-1}\); \(\lambda 95\% \text{ ethanol} \) max 271 \text{ m} \mu, \epsilon 51.

*Anal.* Calcd. for C\(_{12}\)H\(_{18}\)O\(_{12}\)N\(_2\): C, 37.73, H, 4.75; 0, 50.26; N, 7.33; Mol. Wt., 382. Found: C, 37.96, 38.48; H, 4.93, 4.92; 0, 48.75; N, 7.35, 6.94; Mol. Wt., 369.

**Endecaphyllin E.** - With water saturated chloroform, containing 7.0% ethanol endecaphyllin E was obtained (cuts No. 179-191). The isolated tan material, m.p. 122-127\(^\circ\), was recrystallized three times from acetone-chloroform yielding 65 mg. (1.2% yield) of white crystals, m.p. 132-134\(^\circ\); \(\nu \text{ KBr} \) 3450, 1736, 1550, 1392, 1190, 1024, 869 cm\(^{-1}\); \(\lambda 95\% \text{ ethanol} \) max 276 \text{ m} \mu, \epsilon 37.

*Anal.* Calcd. for C\(_{12}\)H\(_{18}\)O\(_{12}\)N\(_2\): C, 37.73; H, 4.75; 0, 50.26; N, 7.33; Mol. Wt., 382. Found: C, 37.43;
\[ \begin{align*}
\text{H, 5.09; O, 49.52; N, 7.81; Mol. Wt., 357.} \\
\text{\(\beta\)-Methyl-D-glucoside.- With 20\% ethanol in water saturated chloroform \(\beta\)-methyl-D-glucoside was isolated (cuts No. 252-266). The naturally occurring glucoside crystallized from ethanol in white plates. It exists in two dimorphic forms of m.p. 92-94°, and 112-114° (lit., m.p. 108040); [\alpha]_D^{22} = -29° (1\% in water, 1 dm.) (lit., -32° 40). The infrared spectrum is indistinguishable from that of an authentic sample. A mixed melting point with an authentic sample (m.p. 114-115°) showed no depression, m.p. 112-115°. A n.m.r. spectrum was taken in D_2O (about 15\% concentration), which shows a doublet at 5.76 \(\tau\) (\(J = 7.4\) c.p.s.), and a singlet at 6.61 \(\tau\). All additional signals are not resolved. Dioxane was used as internal reference standard (\(\tau = 6.43\)).}
\end{align*} \]

\[ \text{Anal. Calcd. for } C_7H_{14}O_6 \cdot \frac{1}{2} H_2O; C, 41.41; H, 7.45; O, 51.23; \text{CH}_3O, 15.26; \text{Mol. Wt., 203. Found: C, 41.51; H, 7.44; O, 50.79; \text{CH}_3O, 15.60; \text{Mol. Wt., 338.} \]

\[ \text{Attempts to eliminate water of crystallization at 100-115°/0.05 mm. over P_2O_5, failed. The compound sublimed under these conditions, but the sublimed material still furnished analytical data for } C_7H_{14}O_6 \cdot \frac{1}{2} H_2O. \]

\[ \text{Anal. Found: C, 41.68; H, 7.32; O, 50.80.} \]

\[ \text{X-ray powder diffraction patterns of the isolated and the sublimed samples were identical. Either the gluco-} \]
side never lost its crystal water or picks it up instantly when exposed to moist air.

A crude sample of β-methyl-D-glucoside was also supplied by Dr. M.P. Morris. The method of isolation is not known.

The total weight eluted during the isolation of all 8 compounds was 5.25 g. (97%).

Molisch Test. - β-Methyl-D-glucoside (3 mg.), isolated from I. endecaphylla, was dissolved in 1 ml. water and 2 drops of a 15% ethanolic α-naphthol solution were admixed. To this mixture 1 ml. of conc. sulfuric acid was added slowly, in order to obtain two phases. A faint purple ring at the phase boundary indicated a carbohydrate.

β-Methyl-D-glucoside-tetraacetate. - β-Methyl-D-glucoside (50 mg.) from I. endecaphylla was dissolved in 1 ml. pyridine and 0.5 ml. acetic anhydride was added. After 24 hrs. at room temperature, the reaction mixture was diluted with water and extracted with ether. The ether extract was washed with 2 N H₂O, water, and dried over magnesium sulfate. Evaporating the ether yielded 65 mg. of the crude tetraacetate, m.p. 102-104°. After three recrystallizations from ether the compound melted at 105-105.5° (lit., m.p. 104-105°). The infrared spectrum shows no hydroxyl absorption. The ester carbonyl absorption appears at 1738 cm⁻¹ (KBr).
Anal. Calcd. for C_{15}H_{22}O_{10}: C, 49.72; H, 6.12; O, 44.16; 4 CH\_3CO-, 47.4. Found: C, 49.56; H, 6.04; O, 44.14; CH\_3CO-, 44.64.

\(\beta\)-Methyl-D-glucoside-tetramethylether. - Using Kuhn's method, 90 mg. of \(\beta\)-methyl-D-glucoside isolated from \textit{I. endecaphylla} was dissolved in 6 ml. dimethylformamide and cooled to 0°. To this solution 0.9 ml. methyl iodide, 600 mg. of barium oxide, and 23 mg. of barium hydroxide were added and stirred at 0° for 18 hrs., and for 14 more hrs. at room temperature. The reaction mixture was then diluted with 30 ml. chloroform, washed with water, then with 10% sodium thiosulfate solution, and again with water. The chloroform was dried over magnesium sulfate and evaporated. A faint yellow oil (66.2 mg) was obtained. Distillation at 40°/0.025 mm. (bulb to bulb) yielded 54 mg. of white crystals, m.p. 32-34° (lit., m.p. 40-41°). The infrared spectrum shows no hydroxyl absorption.

Anal. Calcd. for C_{11}H_{22}O_{6}: C, 52.78; H, 8.86; O, 38.36; 5 CH\_3O-, 62.00; Found: C, 52.54; H, 8.80, O, 38.99, CH\_3O-, 59.7.

\(\beta\)-Methyl-D-glucoside-4,6-\(\alpha\)-benzylidene.-\(\beta\)-Methyl-D-glucoside (36 mg.) form \textit{I. endecaphylla} was dissolved in 2.5 ml. of ethanol and added to a mixture of 21 mg. of benzaldehyde and 10 drops of 6 N hydrochloric acid
(ethanolic). After 48 hrs. at room temperature, the solvent was removed by a nitrogen jet and the resulting white crystalline material dissolved in chloroform. Some insoluble material proved to be starting material. The chloroform solution was washed with water, dried over magnesium sulfate, and evaporated. A white crystalline compound (19.5 mg.), m.p. 196-205°, was obtained (lit., m.p. 199-201°). Two recrystallizations from dioxane-hexane afforded m.p. 160-163.5°; \( \lambda_{\text{max}} \) 95% ethanol (251), 257, (262), (267) \( \mu \mu \), e 198.

**Anal.** Calcd. for \( \text{C}_{14}\text{H}_{18}\text{O}_6 \): C, 59.56; H, 6.43; O, 34.01; Mol. Wt., 282. Found: C, 59.31; H, 6.58; O, 33.80; Mol. Wt., 292.

The change in melting points on recrystallization is difficult to rationalize, however, for \( \alpha \)-methyl-\( \beta \)-glucoside-4,6-0-benzylidene m.p. 161-165° is reported.\(^{40}\) Epimerization due to acid, possibly present during the recrystallization, seems very unlikely, but has to be considered since the \( \beta \)-configuration of the starting material is well established.

In several column chromatography attempts on a larger scale, ethyl-\( \beta \)-nitropropionate and 4 new endecaphyllins were isolated.

**Ethyl-\( \beta \)-nitropropionate.** When 28.4 g. of solvent
free extract was chromatographed on 1750 g. of silica gel (B.D.H.), in the same manner as described before, 300 mg. (1.06% yield) of a yellow liquid was isolated using pure water saturated chloroform as eluent (cuts No. 35-44).

β-Nitropropionic acid came off the column 44 cuts later, while still using the same solvent. Distillation provided a colorless liquid, b.p. 40-50°/0.04 mm. (bulb to bulb); ν<sub>film</sub> 1740, 1555 cm<sup>-1</sup>, λ<sub>max</sub> 95% ethanol 270 μm, e 21.2. A synthetic sample showed b.p. 55°/0.25 mm; ν<sub>film</sub> 1740, 1555 cm<sup>-1</sup>, λ<sub>max</sub> 95% ethanol 270 μm, e 21.1.

The infrared spectrum of the naturally occurring ester is very similar to that of the synthetic sample. Gas chromatographic analysis (113°, 75 ml./min., 20% SP-96 silicone on fire brick) of the naturally occurring and the synthetic material showed identical retention times (15 min.). About 10% of an impurity was present in the naturally occurring ester. The n.m.r. spectrum was taken in deuterochloroform (15% solution). It shows a triplet at 8.73 τ (J = 7.3 c.p.s.) and a quartet at 5.78 τ (J = 7.3 c.p.s.). Furthermore, two triplets at 7.03 τ (J = 5.8 c.p.s.) and at 5.31 τ (J = 5.8 c.p.s.) respectively were observed.

Anal. Calcd. for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N: C, 40.81; H, 6.17; O, 43.50; N, 9.52; Mol. Wt., 147. Found: C, 41.12; H, 6.23;
A solution of 500 mg. \(\beta\)-nitropropionic acid in 15 ml. of ethanol (absolute), and 5 g. of silica gel (B.D.H.) was stirred for 12 days at room temperature. The silica gel was then filtered off and the solvent removed at normal pressure. The semisolid residue was dissolved in ether and several times extracted with 1 N sodium bicarbonate solution, washed with water, and dried over magnesium sulfate. Only 20 mg. (4% yield) of ethyl-\(\beta\)-nitropropionate was obtained after removal of the ether. The ester was identified by its infrared spectrum.

**Endecaphyllin I.**—Chromatography of 35.5 g. of solvent free extract on 2000 g. of silica gel (B.D.H.) yielded crude endecaphyllin I, m.p. 128-135°, with 2% ethanol in water saturated chloroform (cuts No. 212-217). This was the first endecaphyllin obtained after the elution of ethyl-\(\beta\)-nitropropionate and \(\beta\)-nitropropionic acid. After 6 recrystallizations from acetone-chloroform-hexane 35 mg. (0.1% yield) of white crystals were obtained, m.p. 139-140.5°; \(\lambda_{\text{KBr max}}^{\text{max}}\) 3571, 1745, 1710, 1575, 1553 (shoulder), 1391, 1198, 877 cm\(^{-1}\); \(\lambda_{\text{95% ethanol max}}\) 276 mp, \(e\) 39.8

**Anal. Calcd.** for \(C_{16}H_{26}O_{12}N_{2}\): C, 43.87; H, 6.00; O, 43.84; N, 6.40; Mol. Wt., 438. Found: C, 43.93, 43.75; H, 5.68; 5.49; O, 43.44, 44.45; N, 6.05, 6.95; Mol. Wt., 386.
Endecaphyllin A<sub>2</sub>. - From 14.2 g. of solvent free extract, chromatographed on 900 g. of silica gel (B.D.H.) endecaphyllin A<sub>2</sub>, m.p. 150-155°, was eluted as tan crystals with 2.5% ethanol in water saturated chloroform (cuts No. 203-212). The compound was eluted after endecaphyllin A and before endecaphyllin B was obtained. Six recrystallizations from acetone-ethanol-chloroform afforded 7 mg. (0.05% yield) of white crystals, m.p. 156.5-158.5°; $\nu_{\text{KBr}}^{\text{max}}$ 3490 (split), 1725 (split), 1550, 1385 (split), 1195 (split), 1045 (split), 870 cm<sup>-1</sup>. A mixed melting point with endecaphyllin C (m.p. 155-156°) was depressed, m.p. 137-140°. Anal. Calcd. for C<sub>15</sub>H<sub>21</sub>O<sub>15</sub>N<sub>3</sub>: C, 37.30; H, 4.38; N, 8.70; Found C, 37.38; H, 4.20; N, 8.88

Endecaphyllin B<sub>1</sub>. - This compound was isolated on the same column as endecaphyllin A<sub>2</sub> (cuts No. 234-249, 2.5% ethanol in water saturated chloroform). Its elution began one cut after endecaphyllin B and ended 35 cuts before endecaphyllin C (3% ethanol in water saturated chloroform) was obtained. When recrystallized 3 times from acetone-chloroform 95 mg. (0.67% yield) of white crystals were afforded, m.p. 129-130°; $\nu_{\text{KBr}}^{\text{max}}$ 3560, 3310 (broad), 1763 (shoulder), 1740, 1558, 1370, 1272, 1093, 870 cm<sup>-1</sup>; $\lambda_{\text{max}}^{95\% \text{ ethanol}}$ 275 μm, ε 75.5.
Anal. Calcd. for C_{15}H_{21}O_{15}N_3: C, 37.30; H, 4.38; O, 49.69; N, 8.70; Mol. Wt., 483. Found: C, 37.68, 38.54; H, 4.43, 4.45; O, 48.62, 47.33; N, 8.49, 12.47; Mol. Wt., 473.

Endecaphyllin \( C_1 \)- On 800 g. of silica gel (B.D.H.) 10.6 g. of solvent free extract was chromatographed. With 3% ethanol in water saturated chloroform (cuts No.332-341) tan crystalline endecaphyllin \( C_1 \), m.p. 140-145\(^{\circ}\), was obtained immediately after the elution of endecaphyllin \( C \). Three recrystallizations from ethanol afforded 73 mg. (0.76% yield) of white crystals, m.p. 145-146.5\(^{\circ}\);

\[ \nu_{\text{KBr}} \max 3450, 1745, 1715 \text{ (shoulder)}, 1555, 1382 \text{ (split)}, 1182, 1015, 870 \text{ cm}^{-1}. \lambda \text{ 95% ethanol 274 \mu, e 56.5. A mixed melting point with endecaphyllin D (m.p. 145-146\(^{\circ}\)) was depressed, m.p. 126-137\(^{\circ}\). Furthermore, a comparison of the infrared spectra of endecaphyllin \( C_1 \) and D showed them to be very similar, however, there are differences.

Anal. Calcd. for C_{12}H_{18}O_{12}N_2: 37.73; H, 4.75; O, 50.26; N, 7.33; Mol. Wt., 382. Found: C, 37.93; H, 4.80; N, 7.47; Mol. Wt., 475.

Endecaphyllin \( X \)- This compound was supplied by Dr. M.P. Morris\textsuperscript{35} as a brown solid, m.p. 96-100\(^{\circ}\). It was never found during the course of this work. Treatment with charcoal in acetone, and three recrystallizations from acetone-hexane afforded white crystals, m.p.
104-105.5°; ν KBr 3495 (broad), 1748, 1555, 1375, 1175, 1090, 872 cm\(^{-1}\); \(\lambda_{95\%}\) ethanol 274 μ, ε 99.

Anal. Calcd. for C\(_{18}\)H\(_{24}\)O\(_{18}\)N\(_4\): C, 36.99; H, 4.14; O, 49.28; N, 9.59; Mol. Wt., 584. Found: C, 36.72, 39.12; H, 4.40; 4.40; O, 47.54; N, 9.11; Mol. Wt., 576.

In view of the variety of β-nitropropionyl esters isolated, the possibility of acyl migration and disproportionation on the column exists.

Characterization.

Hydrogenation.- The microhydrogenations were carried out on 3-5 mg. of compound in a thermostated room at approximately 10° and atmospheric pressure. In the calculation of the hydrogen uptake, the vapor pressure of the solvent, atmospheric pressure, and temperature were accounted for. The experiments listed in the Table VIII were conducted in order to find a suitable system for the complete and quantitative hydrogenation of the nitro groupings present in the endecaphyllins.
### TABLE VIII

Reduction of Nitro Groupings.

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<th>Compound</th>
<th>Catalyst(a)</th>
<th>Solvent</th>
<th>Time H₂-Uptake</th>
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</thead>
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<td>EtOH</td>
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<td>3.0</td>
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<tr>
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<td>Pd/C</td>
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<td>48</td>
<td>2.1</td>
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<td>0</td>
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<tr>
<td></td>
<td>Pt/C(b)</td>
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<td>2.7</td>
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<td>EtOH</td>
<td>12</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>PtO₂</td>
<td>EtOH</td>
<td>8</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Pt/C</td>
<td>AcOH</td>
<td>18</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>PtO₂</td>
<td>AcOH</td>
<td>27</td>
<td>2.4</td>
</tr>
<tr>
<td>Cinnamic acid(c):</td>
<td>Pd/C</td>
<td>EtOH</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Pd/C</td>
<td>EtOH</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Methyl-β-nitropropionate:</td>
<td>Pt/C</td>
<td>EtOH</td>
<td>15</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Pt/C(d)</td>
<td>EtOH</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>Pt/C(d)</td>
<td>NaOH</td>
<td>1.5</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Pt/C(d)</td>
<td>NaOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pt/C</td>
<td>EtOH/AcOH</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Pt/C(e)</td>
<td>EtOH</td>
<td>4</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Pt/C(e)</td>
<td>EtOH</td>
<td>3</td>
<td>3.0</td>
</tr>
</tbody>
</table>

(a) In each case the catalyst was prehydrogenated.
(b) One drop of 60% aqueous perchloric acid was added to the solution.
(c) The hydrogenation of cinnamic acid was carried out on 15 mg. amounts and the accuracy of the results proved that the erratic hydrogen uptake on the nitrocompounds was not due to small changes in conditions or the apparatus.
(d) One drop of 40% aqueous sodium hydroxide was added to the solution.
(e) An ethanol stock solution containing 0.05% v/v triethylamine was used as a solvent.
Hydrogenation of the Endecaphyllins.- The triethylamine catalyzed hydrogenation of ethyl-β-nitropropionate appeared to be most promising and was therefore employed in the hydrogenation of the endecaphyllins. All reductions listed below were carried out in 3 ml. ethanol (abs.) containing 0.05% (v/v) triethylamine and 10 mg. of 5% Pt on carbon as a catalyst. The solvent containing the catalyst was prehydrogenated.
### TABLE IX

**Hydrogenation of the Endecaphyllins.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount (mg)</th>
<th>Ppm</th>
<th>t°C</th>
<th>Time (hrs)</th>
<th>H₂-Uptake (mole equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.0</td>
<td>742</td>
<td>9</td>
<td>6.5</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>747</td>
<td>9</td>
<td>9.0</td>
<td>8.3*</td>
</tr>
<tr>
<td>B</td>
<td>2.8</td>
<td>747</td>
<td>10</td>
<td>2.2</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>2.8</td>
<td>747</td>
<td>10</td>
<td>4.0</td>
<td>8.9</td>
</tr>
<tr>
<td>B₁</td>
<td>3.5</td>
<td>751</td>
<td>25</td>
<td>7.2</td>
<td>9.2</td>
</tr>
<tr>
<td>C</td>
<td>3.4</td>
<td>745</td>
<td>10</td>
<td>4.3</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>745</td>
<td>10</td>
<td>7.0</td>
<td>9.1</td>
</tr>
<tr>
<td>C₁</td>
<td>3.9</td>
<td>752</td>
<td>24</td>
<td>5.2</td>
<td>6.1*</td>
</tr>
<tr>
<td>D</td>
<td>3.0</td>
<td>745</td>
<td>9</td>
<td>3.0</td>
<td>5.7*</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>750</td>
<td>10</td>
<td>4.0</td>
<td>5.7</td>
</tr>
<tr>
<td>E</td>
<td>3.4</td>
<td>745</td>
<td>10</td>
<td>3.7</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>745</td>
<td>10</td>
<td>6.5</td>
<td>5.8</td>
</tr>
<tr>
<td>X</td>
<td>5.0</td>
<td>742</td>
<td>13</td>
<td>1.1</td>
<td>12.7*</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>745</td>
<td>10</td>
<td>2.0</td>
<td>12.4*</td>
</tr>
<tr>
<td></td>
<td>2.8</td>
<td>745</td>
<td>11.5</td>
<td>0.7</td>
<td>10.8*</td>
</tr>
</tbody>
</table>

(a) Not enough material was available for the hydrogenation of endecaphyllin I; A₁, A₂.

(b) The reported values are corrected by taking the vapor pressure of the solvent in account.

(c) The values are calculated for complete reduction of all the nitro groups in the molecule.

* In all the hydrogenation experiments the uptake was plotted versus time. The values provided with a star are inflection points on the curve where the pace of hydrogen uptake became significantly slower. The final uptakes were considerably higher than the calculated values.
Color Test for Primary and Secondary Nitro Grouping.

Endecaphyllin A (1 mg.) was dissolved in a few drops of dimethylformamide. About 10 drops of 10% aqueous sodium nitrite, the same amount of 10% aqueous sodium hydroxide, and approximately 0.1 ml. carbon tetrachloride was added. The mixture was slowly neutralized with 10% sulfuric acid. At pH 7-9 a yellow-brown color appeared indicating the presence of a -CH$_2$NO$_2$ grouping (blue-green color indicated R$_2$CHNO$_2$). Upon acidifying with glacial acetic acid to approximately pH 4 the color disappeared.

All endecaphyllins were submitted to this test and showed the presence of primary nitro groupings.

Monoacetate of Endecaphyllin A.- A sample of 50 mg. of endecaphyllin A was dissolved in 1 ml. glacial acetic acid and 1 ml. acetic anhydride at room temperature. A drop of 20% aqueous perchloric acid was added to the solution, while cooling it under running water. The mixture remained for 20 min. at room temperature and was subsequently poured into 20 ml. water. An oily emulsion was formed from which the oil settled on standing for 3 hrs. in the refrigerator. The aqueous phase was decanted, the oil dissolved in methanol and a few drops of acetone, dried over MgSO$_4$, and the solvent removed under vacuum. A faint yellow oily
residue (59 mg.) was obtained, which afforded on crystallization from acetone/methanol 41 mg. of white needles, m.p. 124.5-126.5°; \(\text{KBr} \quad 3550, 1748, 1555, 1371, 1169, 1080, 869 \text{ cm}^{-1}\].

Anal. Calcd. for \(C_{17}H_{23}O_6N_3\): C, 38.89; H, 4.42; O, 48.76; N, 8.00; Mol. Wt., 525. Found: C, 38.57; H, 4.47; O, 48.10; N, 9.06; Mol. Wt., 444.

Further recrystallizations did not change the melting point. From the mother liquors another 5 mg., m.p. 122-125.5°, was isolated.

A second attempt to acetylate endecaphyllin A using again glacial acetic acid-acetic anhydride-perchloric acid yielded only the monoacetate. When the monoacetate was treated again, using the same system, no diacetate was obtained. In order to attempt acetylation in a basic medium 10 mg. of endecaphyllin A was dissolved in 0.25 ml. of pyridine and 0.2 ml. acetic anhydride added. After standing at room temperature for 24 hrs. the reaction mixture was poured onto ice and extracted with chloroform. The chloroform was washed several times with water, dried over \(\text{MgSO}_4\), and the chloroform distilled off under vacuum. A yellow oil (11 mg.) was obtained, which yielded on treatment with charcoal a very hygroscopic
glassy film. Its infrared spectrum showed that the material was very crude (the endecaphyllins are very susceptible to base) but had the general features of the starting material.

**Methylation Attempt on the Monoacetate.** - The monoacetate (10 mg.) of endecaphyllin A was dissolved in a few drops of acetone and 0.5 ml methanol. An ethereal solution of diazomethane was slowly added, till the reaction mixture remained yellow. After 15 min. the formed polymethylene was filtered off. Evaporating the filtrate under vacuum, afforded a yellow semisolid. Its infrared spectrum is indistinguishable with that of the starting monoacetate. The successful methylation of the hydroxyl at C₁ on some carbohydrates with diazomethane is reported not to work in all cases.

**Hydrolysis of Endecaphyllin A.** - A sample of 55 mg. of endecaphyllin A was heated in 4 ml. water on the steambath for 1 hr. After 45 min. the compound was dissolved completely. The solution was then evaporated to dryness under a nitrogen jet yielding a semisolid residue which was dissolved in ethanol. The solution crystallized at room temperature affording 45 mg. of white crystals, m.p. 109-110⁰. A mixed melting point with endecaphyllin A (m.p. 120-122⁰) was 110-120⁰. Its
infrared spectrum is indistinguishable with that of endecaphyllin A. The mother liquor, a yellow oil, yielded from acetone-chloroform a few crystals of nitro-propionic acid, m.p. 65-67°. A mixed melting point with an authentic sample showed no depression (m.p. 65-67°). Paper chromatography of the mother liquor showed no glucose.

When a 1 mg. sample of endecaphyllin A was boiled in water for 30 min. and the water evaporated under a nitrogen jet, a yellow oil was obtained. This oil formed very few crystals in ethanol-water, m.p. 101.5-104°. A mixed melting point with endecaphyllin X was strongly depressed (65-88°).

Chromatography on Whatman No.3 paper in 1-butanol-acetic acid-water (4:1:1) showed: Rf 0.65 for endecaphyllin A; Rf 0.69 for the compound, m.p. 101-104°; and Rf 0.74 for the mother liquor. A sample of β-nitropropionic acid showed Rf 0.76.

Acid Hydrolysis of the Endecaphyllins.- Each of the endecaphyllins (1 mg.) was hydrolyzed in 1.2 N HCl on the steambath for 2 hrs. After 5-10 min. the compounds went into solution. When the hydrochloric acid was removed by a nitrogen jet the remaining brown residue was dissolved in a few drops of ethanol-water.
Some of the resulting solution was spotted on paper (Whatman No.3 and horizontally chromatographed with 1-butanol-water-glacial acetic acid (4:1:1). The chromatogram was developed by drying it at room temperature, spraying with 0.1 M silver nitrate solution (ethanol-water), again drying at room temperature and subsequently spraying with 2.5 M KOH in ethanol-water. Dark spots developed instantly. In order to prevent the chromatograms from darkening completely, they were rinsed in an aqueous sodium thiosulfate solution and then dried at 100°. The Rf values in Table X were calculated for the hydrolysis products.

**TABLE X**

**Paper Chromatographic Analysis of the Hydrolysates of the Endocaphyllins.**

<table>
<thead>
<tr>
<th>Endocaphyllins</th>
<th>Rf values</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.05; 0.18; 0.32; 0.92* 0.97*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.05; 0.18; 0.24; 0.32; 0.78; 0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.05; 0.18; 0.24; 0.32; 0.78; 0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.05; 0.18; 0.24; 0.32; 0.78; 0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.05; 0.18; 0.32; 0.78; 0.92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dextrose 0.18; 
β-Nitropropionic acid 0.78 
I 0.03; 0.17; 0.32; 0.78; 
A1 0.03; 0.17; 0.32; 0.77; 
A2 0.03; 0.17; 0.32; 0.77; 
B1 0.03; 0.17; 0.32; 0.77; 
C1 0.03; 0.17; 0.24; 0.33; 0.78; 0.92; 0.97* 
X 0.03; 0.17; 0.34; 0.78; 0.92; 0.97* 
Dextrose 0.17; 0.32; 0.78; 0.92; 0.97*

*These spots were only recognized by their fluorescence under the ultraviolet lamp.
The spots for the $\beta$-nitropropionic acid (0.78) were very faint and their absence in several cases is no indication for the absence of the acid. In general the spots at Rf 0.18 (glucose) and Rf 0.32 were the largest and most intensive.

**Thin Layer Chromatography of the Endecaphyllins.**

Towards the end of this work silica gel (Woelm; no binder) became available. This new silica gel afforded somewhat better separations between the individual endecaphyllins with less streaking.

**TABLE XI**

Rf Values for the Pure Endecaphyllins.

<table>
<thead>
<tr>
<th>Endecaphyllins</th>
<th>Rf values *</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.87</td>
</tr>
<tr>
<td>A</td>
<td>0.77</td>
</tr>
<tr>
<td>A1</td>
<td>0.76</td>
</tr>
<tr>
<td>A2</td>
<td>0.75</td>
</tr>
<tr>
<td>B</td>
<td>0.68</td>
</tr>
<tr>
<td>B1</td>
<td>0.69</td>
</tr>
<tr>
<td>C</td>
<td>0.75</td>
</tr>
<tr>
<td>C1</td>
<td>0.52</td>
</tr>
<tr>
<td>D</td>
<td>0.56</td>
</tr>
<tr>
<td>E</td>
<td>0.49</td>
</tr>
<tr>
<td>X</td>
<td>0.78</td>
</tr>
</tbody>
</table>

*The Rf values are not very well reproducible since some streaking occurs.*
In the chromatography above (Table XI) 1-butanol, acetic acid, water (4:1:1) proved to be most suitable as a solvent system. The plates were developed by drying at room temperature, followed by spraying with conc. sulfuric acid, and subsequent heating for 30 min. at 110°.

**Base Hydrolysis of Endecaphyllin B₁.** - A 1 mg. sample of endecaphyllin B₁ was dissolved in 0.2 ml. of acetone and 0.2 ml. of 0.1 N sodium hydroxide was added. The solution turned yellow instantly. After 30 min. the solution was neutralized with dry ice and the solvent removed under a nitrogen jet. A yellow residue was obtained, which dissolved partially in a few drops of ethanol. Some of the ethanol solution was spotted on a silica gel thin layer (Woelm, no binder) and chromatographed in 1-butanol, glacial acetic acid, ether, water (9:6:3:1). The plate was then developed by drying at room temperature, followed by spraying with conc. sulfuric acid, and subsequent heating for 15 min. at 110°. The observed Rf value of 0.18 was identical with the one of dextrose.

Similar treatment of a sample of tetra-β-nitropropionyl-methyl-β-D-glucoside furnished a spot identical with one obtained from β-methyl-D-glucoside.
(Rf 0.27).

**Base Hydrolysis of Endecaphyllin I.** When endecaphyllin I was treated and chromatographed, as described above for endecaphyllin B₁, glucose was detected (Rf 0.17).

**Treatment of β-Nitropropionic Acid with Strong Base.**
To a sample of 119 mg. (1 mmole) β-nitropropionic acid (placed in a closed test tube with a gas in- and outlet) 2 ml. of 40% aqueous sodium hydroxide was added. The solution was heated for 30 min., while a slow nitrogen stream was employed to carry the generated ammonia into 10 ml. of 0.1 N sulfuric acid. On titration of the excess acid with 0.1 N sodium hydroxide 0.43 mmole (43%) of ammonia was found. In a qualitative experiment the ammonia was detected by its odor.

**Saponification Equivalents of the Endecaphyllins.**
Approximately 5 mg. of each of the endecaphyllins was dissolved in 1 ml. acetone and 1 ml. 0.1 N sodium hydroxide added. The solution turned yellow instantly. After 30 min. the reaction mixture was titrated with 0.01 N sulfuric acid using phenolphthalein as indicator.

The molar equivalents of sodium hydroxide used up are twice as many as expected for the ester hydrolysis. From the data obtained on β-nitropropionic acid, however, it becomes evident that the sodium salt of the
aci-nitro form is formed.

\[ \text{\( \text{O}_2\text{N-CH}_2\text{-CH}_2\text{-CO}_2\text{H} + 2 \text{NaOH}\rightarrow \text{Na}_2\text{N=CH-CH}_2\text{CO}_2\text{Na} + 2 \text{H}_2\text{O} \)} \]

After reaching the neutralization point (titration of excess base), the aci-form will shift slowly (several hrs.) to the nitro-form, when the base generated thereby is continuously neutralized.

**TABLE XII**

**Saponification Equivalents.**

<table>
<thead>
<tr>
<th>Endecaphyllins*</th>
<th>Amount 0.01N H(_2)SO(_4)</th>
<th>0.1N NaOH used up mg.</th>
<th>ml.</th>
<th>ml.</th>
<th>mol.equiv.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.1</td>
<td>3.65</td>
<td>0.635</td>
<td>6.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>4.05</td>
<td>0.595</td>
<td>5.86</td>
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<tr>
<td>A(_1)</td>
<td>3.1</td>
<td>6.05</td>
<td>0.395</td>
<td>6.16</td>
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</tr>
<tr>
<td>B</td>
<td>5.2</td>
<td>3.25</td>
<td>0.675</td>
<td>6.28</td>
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</tr>
<tr>
<td></td>
<td>5.5</td>
<td>3.10</td>
<td>0.690</td>
<td>6.06</td>
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</tr>
<tr>
<td>B(_1)</td>
<td>4.8</td>
<td>4.10</td>
<td>0.590</td>
<td>5.96</td>
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</tr>
<tr>
<td>C</td>
<td>5.0</td>
<td>3.60</td>
<td>0.640</td>
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<tr>
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<td>3.85</td>
<td>0.615</td>
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<tr>
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<td>5.0</td>
<td>4.00</td>
<td>0.600</td>
<td>5.82</td>
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<tr>
<td>C(_1)</td>
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<td>4.45</td>
<td>0.555</td>
<td>4.08</td>
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<tr>
<td></td>
<td>4.5</td>
<td>5.30</td>
<td>0.470</td>
<td>3.99</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>4.3</td>
<td>5.85</td>
<td>0.415</td>
<td>3.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>5.90</td>
<td>0.410</td>
<td>3.91</td>
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</tr>
<tr>
<td>E</td>
<td>3.8</td>
<td>5.95</td>
<td>0.405</td>
<td>4.08</td>
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</tr>
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<td></td>
<td>4.1</td>
<td>5.70</td>
<td>0.430</td>
<td>4.02</td>
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</tr>
<tr>
<td>X</td>
<td>5.2</td>
<td>2.90</td>
<td>0.710</td>
<td>8.01</td>
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</tr>
<tr>
<td></td>
<td>4.9</td>
<td>3.15</td>
<td>0.685</td>
<td>7.98</td>
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<tr>
<td>(\beta)-Nitropro-</td>
<td>6.0</td>
<td>0.50</td>
<td>0.950</td>
<td>1.90</td>
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<tr>
<td>pionic acid</td>
<td>6.0</td>
<td>0.25</td>
<td>0.975</td>
<td>1.95</td>
<td></td>
</tr>
<tr>
<td>Ethyl-(\beta)-</td>
<td>6.0</td>
<td>0.60</td>
<td>0.940</td>
<td>1.73</td>
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</tr>
<tr>
<td>nitro propionate</td>
<td>6.0</td>
<td>0.75</td>
<td>0.925</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>Tetra-(\beta)-</td>
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<td>2.00</td>
<td>0.800</td>
<td>8.03</td>
<td></td>
</tr>
<tr>
<td>propionyl -(\beta)-</td>
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<tr>
<td>methyl-D-gluco-</td>
<td>6.0</td>
<td>1.94</td>
<td>0.806</td>
<td>8.08</td>
<td></td>
</tr>
<tr>
<td>side</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Endecaphyllin I and A\(_2\) were not available in sufficient quantity.
Methylation of Endecaphyllin C with Methyl Iodide.—

In order to investigate the stability of β-nitropropionate under base catalyzed methylation conditions, 100 mg. of ethyl-β-nitropropionate was dissolved in 6 ml. of dimethylformamide (freshly distilled from calcium hydride). To this solution 600 mg. of barium oxide was added, and the suspension stirred at 0° for 18 hrs. Then stirring was continued at room temperature for 14 more hrs., whereafter the reaction mixture was diluted with 30 ml. of chloroform and the barium salts filtered off. The chloroform solution was washed extensively with water, dried over magnesium sulfate, and the solvent removed at 50°/10 mm. A yellow liquid was obtained, the infrared spectrum shows all the features of the starting material with some weak bands attributable to dimethylformamide.

According to the method of Kuhn, 49 50 mg. of endecaphyllin C was dissolved in 3 ml. dimethylformamide (freshly distilled from calcium hydride), the solution cooled to 0° and 100 mg. of barium oxide, 4 mg. of barium hydroxide, and 0.1 ml. of methyl iodide were added. This mixture was stirred for 15 hrs. at 0° and for additional 20 hrs. at room temperature. During the period of stirring the mixture turned gradually
brown. Twenty milliliter of chloroform were then added and the barium salts removed by filtration. The chloroform solution was washed 2 times with 10 ml. water, once with 10 ml. of 10% aqueous sodium thiosulfate and 4 additional times with a total of 40 ml. water, dried over magnesium sulfate, and the solvent removed in vacuo. A black tar (26 mg.) was obtained.

The same procedure applied to β-methyl-D-glucoside yielded 65% of the fully methylated glucoside.

**Preparation of 0.5 M Diazomethane Solutions in Methylene Chloride.** - In a 500 ml. Claisen flask equipped with dropping funnel Liebig condenser and a receiver cooled in dry ice (all connections with rubber stoppers) 24 g. of sodium hydroxide was dissolved in 20 ml. water. When the sodium hydroxide solution was cooled down to room temperature, 50 ml. of 2-methoxyethanol and 50 ml. methylene chloride were added. This mixture was stirred with a magnetic stirrer and cooled to 0°. To the cold solution, 7.1 g. of N,N'-dinitroso-N, N'-dimethylterephthalamide (EXR-101, Dupont) was added in one portion with an additional 50 ml. of methylene chloride. The additional methylene chloride was necessary to facilitate stirring since the mixture became viscous. The tem-
perature was then slowly raised to 50-60° (water-bath) and the methylene chloride-diazomethane co-distilled. After 50 ml. of distillate the methylene chloride came over colorless, at which time the distillation was stopped. The methylene chloride-diazomethane distillate was dried over potassium hydroxide in the ice box, filtered, and analyzed.

**Analysis of the Diazomethane Solution.**—Benzoic acid (122 mg.) was suspended in 10 ml. ether and 1 ml. of the diazomethane solution added. After 10 min. the mixture was diluted with water and titrated under vigorous swirling with 0.1 M NaOH, using phenolphthalein as indicator. The solutions of diazomethane were approximately 0.7 molar.

**Boron Trifluoride-Etherate Stock Solution.**—Freshly distilled (b.p. 127°) boron trifluoride-etherate (0.114 ml.) was added to 10 ml. of dry methylene chloride-ether 1:1.

**Methylation of Tris β-nitropropionyl-methyl-β-D-glucoside with Diazomethane.**—A synthetic sample (p.100) of tris-β-nitropropionyl-methyl-β-D-glucoside (100 mg. 0.2 mmole) was suspended in 5 ml. of dry methylene chloride and 0.1 ml. of the boron trifluoride catalyst stock solution (see above) added. To this
mixture, 6 ml. of a 0.55 M diazomethane solution in methylene chloride (3.3 mmole) were added from a pipette at a flow rate of 2 ml./min. At intervals, during the addition of diazomethane an additional 0.3 ml. of catalyst stock solution were injected in 0.1 ml. portions. During the addition of diazomethane, nitrogen was evolved and polymethylene precipitated as a cloudy white material whereas the glucoside, which can be distinguished from the polymethylene, went slowly into solution. The reaction mixture was then diluted with another 5 ml. of methylene chloride, freed from polymethylene by filtration, washed 3 times with water (20 ml. total), dried over magnesium sulfate and the solvent evaporated in vacuo. A faint yellow oil (99.7 mg) was obtained. Its infrared spectrum showed no hydroxyl band. Crystallization from acetone-chloroform-hexane afforded 85 mg. of white crystals, m.p. 126-127.5°. Recrystallization raised the melting point only a little to 126.5-128°; $\nu_{\text{KBr}}^\text{max}$ 3525 (weak), 1745 (split), 1550, 1376, 1188, 874 cm$^{-1}$.

Anal. Calcd. for $C_{17}H_{25}O_{15}N_3$: C, 39.95; H, 4.93; O, 47.00; N, 8.22; Mol. Wt., 511. Found: C, 39.54; H, 4.74; N, 8.45.

Base hydrolysis of the above described product
and chromatography, as described for the endecaphyllin B, (p. 80), showed Rf 0.34 for the hydrolysate. A sample of β-methyl-D-glucoside which was chromatographed on the same thin layer had Rf 0.28.

Treatment of tetra-β-nitropropionyl-methyl-β-D-glucoside in the same manner as described above gave only starting material back, which indicates the stability of the β-nitropropionylester groupings under these conditions.

**Methylation of Endecaphyllin C in Methylene Chloride.**—A 100 mg. sample of endecaphyllin C (0.205 mmoles) was suspended in 5 ml. of dry methylene chloride. After addition of 0.1 ml. of boron trifluoride catalyst stock solution, 20 ml. of a 0.66 molar diazomethane-methylene chloride solution (13.2 m mole) were dropped into the suspension at a rate of 2 ml./min. In 0.1 ml. portions, 1.0 ml. of catalyst stock solution was added at intervals during the methylation. The endecaphyllin C did not appear to go into solution in appreciable amounts while being treated with diazomethane. The reaction mixture was diluted with 5 ml. methylene chloride and the insoluble material together with the formed polymethylene was filtered off. Then the filtrate was washed several times with a total of
15 ml. of water, dried over MgSO₄, and the solvent evaporated in vacuo to give 30.4 mg. of a faint yellow oil. Its infrared spectrum still shows a hydroxyl band, however, it is weaker than in the starting material. Chromatography of the yellow oil on thin layers of silica gel (Woelm, no binder) with 1-butanol-glacial acetic acid-water (4:1:1) and developing with conc. H₂SO₄, followed by heating for 10 min. at 110°, showed 3 components (Rf 0.70; 0.75 and 0.93). Endecaphyllin C, spotted on the same plate had Rf 0.76. However, the component of Rf 0.75 present in the yellow oil is not identical with endecaphyllin C, (see paper chromatography p.91). Attempts to crystallize this oil failed. Column chromatography on 1 g. of silica gel with iso-hexane-chloroform, and chloroform-ethanol as eluent did not yield any crystalline fractions. The oily fractions obtained, showed still similar mixtures on thin layers (Total recovery 17.5 mg.).

The solid material from filtration of the reaction mixture was heated in 5 ml. of acetone, and the acetone insoluble polymethylene removed by filtration. On evaporating the solvent a colorless oil was obtained, which afforded on crystallization from acetone-chloroform 67.5 mg. of white crystals, m.p. 135-149°. Its
infrared spectrum is indistinguishable from that of the starting material and the melting point was raised by two more recrystallizations to that of endecaphyllin C (150-152°). A mixed melting point showed no depression.

The recovered starting material (60 mg.) was remethylated as described above (16 ml. 0.66 M diazomethane solution, and 0.8 ml. catalyst stock solution) yielding 19.3 mg. of a yellow wax. Its infrared spectrum is similar to that of the oil obtained above, and thin layer chromatography (as described above) gave identical Rf values (0.70; 0.76 and 0.93). In addition, 38 mg. of starting material was recovered.

These combined oily products (36.8 mg.) were remethylated (5 ml. of 0.6 molar diazomethane solution and 0.4 ml. catalyst stock solution). Again a yellow oil (21.3 mg.) was obtained which showed no change in the infrared spectrum and on thin layer chromatography.

The 38 mg. of recovered starting material (above) afforded on remethylation (6 ml. of 0.6 molar diazomethane and 0.3 ml. of catalyst stock solution) and on work up, 7.9 mg. of a yellow oil. This oil had again a similar infrared spectrum to those of the yellow oils obtained above and identical Rf values on thin layer
chromatography (Rf 0.70; 0.93). The recovery of starting material was 31 mg.

**Methylation of Endecaphyllin C in Tetrahydrofuran.**

A sample of 50 mg. of endecaphyllin was dissolved in 1.5 ml. tetrahydrofuran (freshly distilled from lithium aluminium hydride). The methylation (20 ml. of a 0.4 M diazomethane solution and 0.6 ml. boron trifluoride catalyst stock solution) and the work up was carried out as described before. From the extraction of the polymethylene 5.8 mg. of starting material was obtained. The filtrate (tetrahydrofuran-methylene chloride soluble) furnished 85.8 mg. of a yellow oil the infrared spectrum still shows hydroxyl absorption. The OH absorption appears abnormally strong, for the type of compound expected. Its infrared spectrum and the abnormal weight increase suggests a by-product from the solvent.

Remethylation of the starting material (31 mg.), recovered from the methylation in methylene chloride described above, in tetrahydrofuran (10 ml. of 0.4 M diazomethane solution and 0.5 ml. catalyst stock solution) furnished 51 mg. of a yellow oil which exhibited the same infrared spectrum as the oil (85.8 mg.) obtained above. Both oils were combined and crystalliza-
tion from acetone-chloroform-hexane yielded 54 mg. of a gummy solid, which afforded after 2 further recrystallizations 25 mg. of white crystals, m.p. 98-101°.

\[ \text{KBr} \quad \text{max} \quad 3550, 1736, 1721 \text{ (shoulder), 1555 (split), 1388, 1196, 875, 764 cm}^{-1}. \]

\textbf{Anal.} Calcd. for C\(_{13}\)H\(_{20}\)O\(_2\)N\(_2\): C, 39.43; H, 5.09; N, 7.07; CH\(_3\)O\(^-\), 7.83. Found: C, 39.92; H, 4.48; N, 7.27; CH\(_3\)O\(^-\), 7.09. The compound consumed 4.15 equivalents of base (calcd. 4.00 equiv.).

Repeated crystallizations did not change the melting point.

The crystalline compound (m.p. 98-101°) and the mother liquors obtained from its crystallization were chromatographed on silica gel thin layer (Woelm; no binder) and on paper (Whatman No.3) with 1-butanol, glacial acetic acid, water (4:1:1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rf values (a)</th>
<th>Rf values (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>m.p. 98-101°</td>
<td>0.75</td>
<td>0.82</td>
</tr>
<tr>
<td>Mother liquor</td>
<td>0.75; 0.94; 1.0(8)</td>
<td>0.87; 0.97</td>
</tr>
<tr>
<td>Endecaphyllin C</td>
<td>0.76</td>
<td>0.75</td>
</tr>
</tbody>
</table>

(a) Rf values obtained on silica gel and developed by spraying with conc. H\(_2\)SO\(_4\), followed by heating for 10 min. at 120°.

(b) Rf values obtained on paper and developed with silver nitrate and potassium hydroxide.

(c) Presumably a by-product from tetrahydrofuran.
A 1 mg. sample of the methylation product (m.p. 98-101°) was hydrolyzed in 2 N hydrochloric acid for 1 hr. on the steam bath. The acid was removed under a nitrogen jet, then the residue dissolved in a few drops of ethanol and thin layer chromatographed, using the same system as for the starting material (above). The hydrolysate showed Rf 0.20; a sample of β-methyl-D-glucoside chromatographed on the same plate gave Rf 0.19.

**Synthesis of Some-β-Nitropropionic Acid Derivatives.**

**Preparation of β-Nitropropionic Acid**\(^{39}\).— Freshly distilled β-propiolactone\(^{64}\) b.p. 62°/20 mm, (72 g. = 1 mole) was added over a period of 1 hr. to a solution of 69 g. (1 mole) sodium nitrite in 400 ml. water. During the addition the mixture was stirred and kept at 15-25°. After the addition was completed, stirring was continued for an additional hour, and then the solution acidified to pH 2-3 with conc. HCl. The clear, yellow reaction mixture was continuously extracted with 600 ml. of ether for 50 hrs. During the extraction the aqueous solution turned red-brown and evolved nitrogen dioxide. To the redish ether extract 150 ml. of chloroform was added, the solution treated with charcoal, dried over magnesium sulfate, and the ether removed. From the
remaining chloroform solution 40 g. of β-nitropropionic acid, m.p. 61-65°, crystallized in long, white needles on standing in the refrigerator. Three further recrystallizations from chloroform yielded 32 g. (27% yield), melting at 65.5-67°.

This yield is approximately 10% lower than the one reported and could not always be achieved. In other preparations yields of only 15-20% were obtained. When 58.3 g. (1.1 mole) of ammonium chloride was added to the aqueous sodium nitrite solution, yields of approximately 30% were consistently obtained.

**Ethyl-β-Nitropropanate.** -β-Nitropropionic acid (3 g.) was dissolved in 30 ml. of ethanol (absolute) and a catalytic amount of p-toluene-sulfonic acid was added. The solution was refluxed overnight and the excess ethanol distilled off (normal pressure). The remaining 3.5 g. of yellow liquid were taken up in ether, washed with 1 M sodium bicarbonate solution and water, dried over magnesium sulfate, and the ether removed. Distillation of the residue at 45.5°/0.07 mm. afforded 2.9 g. (78% yield) of a colorless liquid with $\nu_{\text{max}}$ 1740, 1550 cm$^{-1}$ and $\lambda_{\text{max}}$ 270 µµ, $\epsilon_{21.1}$. 

93
Anal. Calcd. for C_{5}H_{9}O_{4}N: C, 40.81; H, 6.17; O, 43.50; N, 9.52; Mol. Wt., 147. Found: C, 41.12; H, 6.23; O, 43.28; N, 7.98; Mol. Wt. 157.

\textit{β-Nitropropionyl Chloride}. - To a sample of 25 g. (0.21 mole) of \textit{β}-nitropropionic acid, 60 ml. (0.8 mole) of thionyl chloride were added and the mixture refluxed under nitrogen for 15 minutes. After a few minutes the acid was completely dissolved. The excess thionyl chloride was removed by distillation at 72°, and the remaining residue fractionated under a nitrogen atmosphere. A main cut of 27 g. (94\% yield) of a colorless liquid distilled at 67°/0.2 mm.; \nu_{\text{film}} 1784, 1555, 1360, 1052, 931, 709 cm\textsuperscript{-1}.

Anal. Calcd. for C_{2}H_{4}O_{3}Cl: Cl, 25.36. Found: Cl, 25.75. The compound consumed 1.98 equivalents of base (calcd. 2.00 equiv.)

\textit{β-Nitropropionic Anhydride}. - \textit{β}-Nitropropionic acid (5.2 g. 44 mmole) was dissolved in 5 ml. of tetrahydrofuran (freshly distilled from lithium-aluminumhydride), and cooled to 0°. The cold solution was stirred and 3 ml. (2.4 g., 44 mmole) of methoxyacetylene was injected with a syringe. A vigorous reaction occurred instantly, whereafter the anhydride crystallized within a few minutes (slow addition of methoxyacetylene
afforded lower yields). The filtered and dried crystals (4.1 g) melted at 59-63°. Two further recrystallizations from tetrahydrofuran afforded 3.8 g. (76% yield) of tan crystals, m.p. 64-65°; \( \nu \) KBr 1817, 1749, 1555, 1371, 1070, 961, 879, 840 cm\(^{-1}\) max.

**Anal. Calcd.** for C\(_6\)H\(_8\)O\(_7\)N\(_2\): C, 32.73; H, 3.66; O, 50.88; N, 12.73. **Found:** C, 32.43; H, 3.70; O, 50.43; N, 13.25.

A mixed melting point with β-nitropropionic acid was strongly depressed (45-50°).

**N-(β-Nitropropionyl)-Piperidine.** To a mixture of 5 ml. piperidine and 20 ml. chloroform, 2.1 g. of β-nitropropionyl chloride were slowly added under stirring at room temperature. During the addition the temperature rose to about 45°. The reaction mixture was stirred overnight at room temperature, then washed with 2 N sulfuric acid, 2 N sodium bicarbonate solution and water, dried with magnesium sulfate and the solvent removed under vacuum. A brown residue of 1.137 g. of mobile oil was obtained which distilled at 120-140°/0.25 mm. (bulb to bulb) as a light yellow oil (990 mg. 35% yield, crystallizing and melting below room temperature). It shows \( \nu \) film max 1647, 1553, 1442, 1250,
1022, 958, 877, 868 cm\(^{-1}\).

**Anal. Calcd. for **C\(_8\)H\(_{14}\)O\(_3\)N\(_2\):** C, 51.60; H, 7.58; O, 25.78; N, 15.04. **Found: C, 51.71; H, 7.73; O, 25.59; N, 14.77.**

**Tetra-\(O\)-(\(\beta\)-Nitropropionyl)-\(D\)-Glucopyranose.**

To a solution of 255 mg. (1.42 mmole) of anhydrous dextrose in 3 ml. of N-methylpyrrolidone (distilled from calcium hydride, b.p. 42-44\(^0\)/25 mm.), 1.16 g. (8.4 mmole) of \(\beta\)-nitropropionyl chloride in 1 ml. of N-methylpyrrolidone were added slowly (30 min.) through a dropping funnel. The reaction mixture was kept under a nitrogen atmosphere, stirred and cooled in an ice bath. After the addition was completed, stirring was continued for 1 more hr. at 0\(^0\), and for an additional 10 hrs. at room temperature. The reaction mixture was then poured on ice and a brown oily layer settled to the bottom. This oily layer was diluted with 30 ml. of chloroform (washed with water, poured over a Woelm alumina column activity I, neutral, and distilled) and the 2 phases separated. The chloroform phase was washed several times with 1 M sodium bicarbonate and water, dried over MgSO\(_4\), and the solvent removed in vacuo. A brown oil (576 mg.) was obtained, which showed a strong amide carbonyl band and a shoulder in
the ester carbonyl region. Pumping off most of the N-methylpyrrolidone at room temperature /0.5 mm.
(15 hrs.) yielded 371 mg. of a brown viscous residue. Its infrared spectrum exhibits equally strong bands at 1750, 1663, 1550 cm\(^{-1}\). An additional 90 mg. with a similar infrared spectrum was obtained by neutralizing the aqueous phase with sodium bicarbonate, extraction with chloroform and applying the same work up as before.

No crystallization of the product could be achieved. Column chromatography of the combined oils (461 mg.) on 20 g. of silica gel (B.D.H.) yielded with 1% ethanol in water saturated chloroform 146 mg. of an oily fraction, which shows both, ester carbonyl (1750 cm\(^{-1}\)) and nitro band (1550 cm\(^{-1}\)) in its infrared spectrum. Crystallization from acetone-chloroform and two further recrystallizations from the same solvents afforded 73.5 mg. (9% yield) of white crystals, m.p. 145-146.5°; \(\upsilon\) \(\text{KBr}\) 3560, 1748, \(\text{max}\)
1552, 1382, 1195, 1030, 868 cm\(^{-1}\); \(\lambda\) \(\text{NO}_2\) 263 \(\mu\)m, e 75. \(\text{max}\)

Anal. Calcd. for \(\text{C}_{18}\text{H}_{24}\text{O}_{18}\text{N}_{4}\): C, 37.02; H, 4.14; N, 9.58. Found: C, 37.20; H, 4.27; N, 9.68. The compound consumed 8.00 equiv. of base (calcd. 8.00 equiv.).

From the mother liquors 24.6 mg., m.p. 142-150°, were isolated. A mixed melting point with endecaphyllin C or D was strongly depressed.
Tetra-O-(β-Nitropropionyl)-β-Methyl-D-Glucoside.

In a 50 ml. 3-neck flask, equipped with a gas inlet tube, a dropping funnel and a dry ice condenser, 1.62 g. C\textsubscript{7}H\textsubscript{14}O\textsubscript{6} (8.35 mmole) was dissolved in 3 ml. of N-methylpyrrolidone. The solution was cooled to 0° and a slow nitrogen stream bubbled through. Over a period of 4 hrs. 5.62 g. (41 mmole) of β-nitropropionyl chloride was added. The outlet of the condenser had been connected to 2 consecutive traps filled with 0.1 N sodium hydroxide and a few drops of phenolphthalein solution. However, it was not possible to follow the course of the reaction by the amount of hydrogen chloride developed during the addition of the acid chloride. The N-methylpyrrolidone apparently tied up the hydrogen chloride completely.

After 1 equivalent of the acid chloride was added, the reaction mixture turned syrupy. An additional 2 ml. of N-methylpyrrolidone had to be added and the temperature allowed to come to 25° in order to maintain the stirring action of the nitrogen jet. Approximately 2 hrs. after the addition was completed the reaction mixture crystallized (yellow needles). These crystals could not be isolated because they liquified as soon as exposed to moist air. Addition of 40 ml. chloroform
to the reaction mixture dissolved the crystals. The brown solution was then poured into 4 g. of sodium bicarbonate in ice water. After further addition of 20 ml. chloroform and separation of the layers, the aqueous phase was extracted several times with chloroform. The combined chloroform extracts were washed with 2 N sodium bicarbonate solution and water, dried over magnesium sulfate, and the solvent removed in vacuo. A viscous, brown oil (3.75 g.) remained. Its infrared spectrum shows ester and amide carbonyl bands, and a nitro band. When crystallization attempts failed, the oil was chromatographed on 250 g. of silica gel (B.D.H.). With 1% ethanol in water saturated chloroform, 1.21 g. of a yellow oil was obtained which showed the nitro ester characteristics in its infrared spectrum. Crystallization and recrystallizations from acetone-chloroform-hexane afforded 936 mg. (18.7%) of white crystals, m.p. 109-110°. KBr

\[ \text{max} \quad 1750, 1555, 1378, 1198, 1058, 872 \text{ cm}^{-1}; \]

\[ \text{max} \quad 265 \text{ mp, e 106.} \]

Anal. Calcd for C\(_{19}\)H\(_{26}\)O\(_{18}\)N\(_4\): C, 38.16; H, 4.38; N, 9.35. Found: C, 38.49; H, 4.48; N, 9.16. The compound consumed 8.01 equivalents of base (calcd. 8.00
With 2% ethanol in water saturated chloroform 850 mg. of oily nitro esters were isolated, which showed a strong hydroxyl band in the infrared spectrum. Rechromatography or crystallization attempts failed to produce crystalline compounds.

Shortly after the oily fractions a white crystalline compound, m.p. 143-146°, came off the column and is described below.

**Tris-O-(β-Nitropropionyl)-β-Methyl-D-Glucoside.**

The compound was recrystallized from acetone-chloroform which afforded 210 mg. (5% yield) of white needles, 144-146.5°; ν\textsubscript{KBr} 3460 (broad), 1748, 1735 (shoulder), 1565 (shoulder), 1555, 1398, 1193, 872 cm\textsuperscript{-1};

\[\lambda_{\text{max}} \text{OH-CN} = 263 \text{mu, } e 88.\]

**Anal. Calcd.** for C\textsubscript{16}H\textsubscript{23}O\textsubscript{15}N\textsubscript{3}: C, 38.66; H, 4.66; N, 8.46. Found: C, 39.12; H, 4.82; N, 8.26. The compound consumed 5.98 equiv. of base (calcd. 6.00 equiv.)

A mixed melting point with endecaphyllin D was depressed (115-130°). An O-methyl derivative was prepared (p. 85).
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Naturally Occurring 4-Phenylcoumarins.

The simplest naturally occurring 4-phenylcoumarins are dalbergin (I) and methyldalbergin (II).\(^1\) They were isolated from the heart wood of Dalbergia sissoo, a North Indian timber wood.

\[\begin{align*}
(I) \quad & R=H \\
(II) \quad & R=\text{CH}_3
\end{align*}\]

Investigation of various Gutiferae species have uncovered several closely related 4-phenylcoumarins\(^2(a)\) which all have phloroglucinol as a building unit in common. Calophyllolide (III) the first among them studied in detail,\(^3(a)-(d)\) was found\(^3(e)\) together with inophyllolide (IV) and callophyllic acid (V) in the fruits of Calophyllum inophyllum, a tropical tree from Asia. A review on the chemistry of these compounds has been published by Polonsky.\(^3(f)\) From Mesua ferrea (family of the Guttiferae) Mesuol (VI) was obtained.\(^4\)
Coumarins Isolated from *Mammea americana* L.

*Mammea americana* L. is a Guttiferae species under consideration in the present investigation. In earlier work the isolation\(^5\) of the insecticidal constituent mammein from the seed oil and its structure (VII)\(^6\) was reported. Mammein was shown to isomerize to isomammein (VIII) in methanolic potassium hydroxide, followed by acidification.\(^7\)

![Chemical structures](image)

Subsequently, a second toxic coumarin was isolated from the peelings of the fruit and shown to possess structure IX.\(^7\) This 4-phenyl-5,7-dihydroxy-6-isovaleryl-8-isopentenylcoumarin was later named mammesin.\(^2(a)\)
Since the *Calophyllum*, *Mesua*, and *Mammea* genera belong to the same family, the similarity of the structures of these 4-phenylcoumarins is not too surprising and phytochemically acceptable. These structures are readily accommodated by current theories on coumarin biogenesis and show no exceptional features. 9, 2(b)
RESULTS AND DISCUSSION.

Isolation.

When a petroleum ether extract of the seeds of *Mammea americana* L. was chromatographed on an aluminum oxide column, using Skelly Solve B - benzene mixtures as eluent, a yellow wax was obtained. Several recrystallizations from hexane afforded a very small amount of white crystals which melted at 148-150°. From its microanalytical data the formula C_{20}H_{34}O was derived. The infrared spectrum (KBr) showed a hydroxyl band (3450 cm^{-1}), the only absorption which could be assigned to a functional group. Therefore, the compound was called mammeol. For lack of material the investigation on this substance was not continued. Later during the chromatography, a partly crystalline yellow oil was obtained among several other yellow oily fractions. Rechromatography of the semi-solid fraction did not furnish a crystalline product. However, a series of up to 30 recrystallizations from ether-hexane afforded yellow needles, m.p. 144-146°, in low yield. This substance was named mammeigin. Very small amounts of this compound had been found earlier in yellow oils obtained from mammea extracts.
Structural Elucidation.

The microanalytical data of this compound were satisfied by the formula \( \text{C}_{25}\text{H}_{24-26}\text{O}_5 \) and might have been isomeric with mammeisin (IX), \( \text{C}_{25}\text{H}_{26}\text{O}_5 \). Infrared bands (KBr) at 773 and 708 cm\(^{-1}\) supported the presence of a monosubstituted phenyl group. The phenolic function was recognized by its characteristic base shift of its ultraviolet maxima and a hydroxyl band (3440 cm\(^{-1}\)) in its infrared spectrum. In addition, the compound gave a green color with ferric chloride. From the definite similarity of the infrared spectrum to that of mammeisin (IX),

\[
\begin{align*}
X & \\
\text{HO} & \\
\text{H} & \\
\text{HO} & \\
\text{CO} & \\
\end{align*}
\]

the compound was at first assumed to be "isomammeisin" (X) in analogy to isomammein (VIII).

However, an exceptionally revealing n.m.r. spectrum led to the rejection of the structure X. The environment of every hydrogen atom in the molecule could be unambiguously defined (Figure 2, and discussion of n.m.r. spectra in the next section). It was possible to deduce
the presence of a isovaleryl group, a 2,2-dimethyl chromene ring, a phenolic hydroxyl, and a phenyl group as substituents in mammeigin. This deduction and the integral of the spectrum (total of 24 H) were consistent with the molecular formula $C_{25}H_{24}O_5$.

The five coumarins previously isolated from the plant family Guttiferae, are all derived from phloroglucinol, and four of them contain a phenyl substituent attached to the 4-position. On phytochemical grounds it was therefore decided to extend these features also to mammeigin. This decision narrowed the possible structures for mammeigin to the following isomers:

\[ \text{XI} \quad \text{XII} \quad \text{XIII} \]
The isomers XI and XII would be derived from a 4-phenyl analog of mammein (VII), and isomer XIII from mammeisin (IX), both obtained and reported\textsuperscript{6,8} earlier from *Mammea americana* L.

It became evident that mammeigin must be represented by structure XIII, when a ready interconversion with mammeisin (IX) through the dehydro derivative XIV, m.p. 164-165°, was effected.
The hydrogenation (a) was carried out in ethanol-tetrahydrofuran at atmospheric pressure, and room temperature with palladized charcoal as catalyst. The cyclization (b) occurred readily in glacial acetic acid containing a trace of sulfuric acid. The identity of the dihydro derivatives obtained from either route a or b was verified by mixed melting point (no depression) and infrared and n.m.r. spectra comparison. Its microanalytical data were in good agreement with the molecular formula C_{25}H_{26}O_5. Infrared bands at 1644 and 753 cm$^{-1}$ can be assigned to the double bond in the chromene ring of mammeigin (XIII), because these bands were not observed in the dihydro derivative XIV.
Discussion of NMR Spectra.

The spectra were run on the Varian Associates A-60 NMR spectrophotometer operated at 60 mc. They were taken with carbon tetrachloride as solvent and the concentrations were about 15%. The chemical shifts are in tau units and the coupling constants in c.p.s., measured against tetramethyl silane as internal reference standard ($\tau = 10.0$).

The n.m.r. spectrum of mammelisin (Fig. 1) shows a total of 26 hydrogens and each signal can be defined. The phenyl hydrogens ($H_a$) appear as a singlet (integral: 5 H) at 2.71T. Two singlets at 0.21 and -0.79T (integral: 1 H for each) are somewhat diffuse and are assigned to the two phenolic hydrogens ($H_{f,k}$). The only other singlet which shows an integral of 1 H appears at 4.30T and must stem from the hydrogen ($H_b$) in the 3-position. This assignment receives further support from the spectra of mammelisin (Fig 2; $H_b$ 4.34T) and dihydromammeligin (Fig. 3; $H_b$ 4.19T). Two singlets at 8.18 and 8.31T (integral: 3 H for each) are assigned to the nonequivalent methyl hydrogens ($H_e$) of the isopentenyl group and a triplet at 4.89T (integral: 1 H) to its vinyl hydrogen ($H_d$), which is split by the two benzyllic hydrogens ($H_c$). The coupling constant cannot be exactly determined, because the two lower peaks of the triplet are not sharp.
FIGURE I

NMR Spectrum of Mammeisin
The benzylic hydrogens ($H_c$) in turn are split by the vinyl hydrogen ($H_d$) and show a doublet at 6.58 T ($J = 8.0$ c.p.s.). Its chemical shift is in good agreement with the reported assignment to the benzylic hydrogens (6.67 T) in 7-acetoxy-6-allyl-4, 8-dimethylcoumarin. $^{12(a), 13}$ A doublet at 9.10 T ($J = 6.4$ c.p.s.) is assigned to the methyl hydrogens ($H_h$) of the isovaleryl group which are split by the methine hydrogen ($H_i$). The assignment is based on the integral for 6 hydrogens and reported chemical shifts for the methyl hydrogens (9.07 T) in methyl isobutyl ketone. $^{12(b)}$

It is also consistent with the findings from mammeigin (9.06 T) and dihydromammeigin (9.05 T). From the methylene hydrogens $H_g$ stems the doublet at 7.19 T, $J = 6.9$ c.p.s. (integral: 2H), which is confirmed by a doublet at 7.14 T ($J = 6.9$ c.p.s.) in mammeigin (Figure 2), because mammeigin does not have any other methylene hydrogens. A broad multiplet appears for the methine hydrogen ($H_i$) from about 7.5 - 8.1 T.

The spectrum of mammeigin (Figure 2) shows tau values, coupling constants, and relative integration values for hydrogen signals of the phenyl hydrogens ($H_a$, 2.94 T), the vinyl hydrogen at the 3-position ($H_b$, 4.34 T), and the hydrogens of the isovaleryl group.
FIGURE II

NMR Spectrum of Mammeigin
(Hf, 7.14 T, J = 6.9 c.p.s.; Hn, 7.72-8.33 T, multiplet; Hg, 9.06 T, J = 6.4 c.p.s.) are in excellent accord with those observed for mammeisin (above). Its phenolic hydroxyl hydrogen (Hd) must be strongly chelated, which accounts for its low tau value (-4.17). Similar low tau values for chelated phenolic hydroxyl hydrogens have been reported. A singlet at 8.48 T (integral: 6 H) can be assigned to the methyl hydrogens (He) of the chromene ring, and two doublets at 4.59 T (J = 10 c.p.s.) and 3.33 T (J = 10 c.p.s.) and 3.33 T (J = 10 c.p.s.) to the vinyl hydrogens Hd and Hc respectively. The integral of each doublet accounts for one hydrogen. These values are in accord with reported findings for 2,2 dimethylchromene systems\(^{12(b)},13,14\) as in jacareubin trimethyl ether\(^{14}\) (8.54, 4.41 and 3.32 T, J = 10 c.p.s.).

In the spectrum of dihydromammeigin (Figure 3) the two doublets observed for Hc and Hd in mammeigin have disappeared. Instead, two triplets appeared for the hydrogens Hc and Hd in the chroman ring. The triplet at 8.13 T (J = 6.8 c.p.s.) stems from the \(\beta\)-hydrogens (Hd) and the one at 7.13 T (J = 6.8 c.p.s.) from the benzylic hydrogens (Hc). For the benzylic hydrogens of the chroman ring in \(\alpha\)-tocopherol\(^{12(d)}\) 7.38 T is reported. The triplet at 7.13 T is partly buried under the doublet for the
FIGURE III

NMR Spectrum of Dihydromammeigin
methylene hydrogens $\left(H^\text{methylene}\right)$ ($7.10 \tau, J = 6.4$ c.p.s.) of the isovaleryl group. This overlap of the signals for these hydrogens is consistent with the relative integral value for 4 hydrogens. The triplet at $8.13 \tau$ for the $H^\text{d}$ appears somewhat diffuse and, in addition, there is overlap in this region by the multiplet of the methine hydrogen $\left(H^\text{nh}, 7.4-8.4 \tau\right)$ integral: 3 H) of the isovaleryl group. The phenolic hydroxyl hydrogen $\left(H^\text{p} \right)$ again shows up at low field ($-4.58 \tau$, integral: 1 H) and is in good agreement with the finding in mammeigin ($-4.17 \tau$). Finally the tau values for the phenyl hydrogen $\left(H^\text{p} \right)$, the vinyl hydrogen on the 3-position $\left(H^\text{v} \right)$, the gem dimethyl hydrogens $\left(H^\text{g}, 8.53 \tau\right)$, the methyl hydrogens $\left(H^\text{m}, 9.05 \tau, J = 6.4$ c.p.s.$\right)$ and the methine hydrogen $\left(H^\text{m}, 7.4 -8.4 \tau\right)$ of the isovaleryl group do not deviate very much from the values observed for mammeigin and mammeisin.
EXPERIMENTS

The infrared spectra were taken on the Perkin-Elmer Model No. 237 spectrophotometer. The Cary Model 14 and the Perkin-Elmer 202 were used to take ultraviolet spectra. Analyses were performed by the Mikroanalytisches Laboratorium im Max-Plank-Institut fuer Kohlenforschung. The melting points were taken on a Fischer-Johns melting point block and are uncorrected.

All solvents used in this work were distilled.
Isolation.

Extraction of 75 lb. of dry seeds of *Mammea americana* L. with Skelly Solve B provided 1.6 kg. of mammey oil after the removal of all acetone insoluble material. Twenty grams of this extract was chromatographed on 1375 g. of aluminum oxide (Merck, acid washed). The column was eluted with Skelly Solve B using an increasing ratio of benzene. Cuts of 500 ml. were taken.

**Mammeol.**—The first crystalline compound, mammeol, was obtained from a yellow wax (cut No.135-151), eluted with Skelly Solve B - benzene (1:1). Several crystallizations of this material from hexane afforded 15 mg. (3.55 \times 10^{-3} \% yield, based on dry seeds) of white crystals, m.p. 148-150°; \( \nu_{\text{max}} \) 3450, 2970, 1455, 1178, 1066, 968, 979 cm\(^{-1}\).

**Anal.** Calcd. for C\(_{20}\)H\(_{34}\)O: C, 82.69; H, 11.80; 0, 5.51; Mol. Wt., 290. Found: C, 82.29, 82.87; H, 11.69, 11.89; O, 5.56, 4.87; Mol. Wt., 320, 367.

**Mammeiglin.**—With Skelly Solve B - benzene (1:9; cuts No.210-219) 237 mg. of a partly crystallized yellow oil was obtained. Separation of the crystalline material from the oil on an aluminum oxide column (Woelm
neutral, activity I) proved futile. Up to 30 crystallizations from ether-hexane afforded 58 mg. (1.37-10^{-2}% yield, based on dry seeds) of yellow needles, m.p. 144-146°; ν \text{KBr} 3440 (broad), 1746, 1644, 1613, 1126, 773, 752, 708 cm\(^{-1}\); λ 95% ethanol 234, 286, 365 μm, max log ε 4.45, 4.52, 4.11; λ 95% ethanol-NaOH 218, 251, 312, 438 μm, max log ε 4.33, 4.38, 4.41, 3.84. The compound gave a green color with ferric chloride.

*Anal.* Calcd. for C\(_{25}\)H\(_{24}\)O\(_5\): O, 74.24; H, 5.98; O, 19.78; Mol. Wt., 404. Found: O, 74.34, 74.04; H, 6.15, 6.34; O, 19.81; Mol. Wt., 401.

The n.m.r. spectrum is reproduced on p. 118. The relative integral is measured as follows: - 4.17τ (1); 2.94 τ (5.4); 3.33 τ (1.1); 4.34 τ (1); 4.59τ (0.8); 7.14 τ (2.1); 7.72- 8.33τ (0.8); 8.48 τ (6.3); 9.06 τ (6.2).

**Dihydromammeigin (XIV) from Mammeigin (XIII).** Mammeigin (50 mg.) was stirred in 3 ml. ethanol with 5% palladium on carbon (10 mg) in a hydrogen atmosphere at 25°. The compound was not completely soluble in the amount of solvent which was limited by the size of the microhydrogenator. After 2 hrs. 0.4 molar equivalents of hydrogen were taken up and further consumption occurred at a very slow pace. Ethanol was removed by a
nitrogen jet and replaced by 3 ml. of tetrahydrofuran (distilled from LiAlH₄). Additional 5 mg. of catalyst were introduced and the hydrogen uptake ceased after twenty minutes. The total uptake was 0.97 molar equivalents. Filtration of the reaction mixture and evaporation of the solvent afforded a yellow semisolid. Crystallization from tetrahydrofuran-hexane yielded 42 mg. of tan crystals, m.p. 152-160°. Three additional recrystallizations raised the melting point to 164-165° (25 mg.). From mother liquors 11 mg., m.p. 154-161°, were obtained. A mixed melting point with a sample of dihydromammeigin, m.p. 165-166°, obtained on cyclization of mammeisin showed no depression, m.p. 164-166°. The infrared spectra of both samples were superposable, and the n.m.r. spectra (Figure 3, p.120) showed identical shifts and coupling constants.

Dihydromammeigin (XIV) from Mammeisin (IX).- A sample of 250 mg. of mammeisin was dissolved in 5 ml. glacial acetic acid and 3 drops of conc. sulfuric acid were added. After standing for 18 hrs. at room temperature, the reaction mixture was poured on to ice-water. A tan amorphous material precipitated. The filtered solid was dissolved in ether, washed several times with
water, and dried over magnesium sulfate. Removal of the ether afforded 233 mg. of tan crystals, m.p. 146-158°. Three further recrystallizations from tetrahydrofuran-ether-hexane yielded tan needles, m.p. 165-166°;

$\gamma_{\text{KBr}}^{\text{max}}$ 3436 (broad), 1745, 1730 (shoulder), 1613, 1587 (shoulder), 1124, 770, 714 cm$^{-1}$; $\lambda$ 95% ethanol 287, $\lambda_{\text{max}}$ 341 μ, log ε 4.54, 4.05; $\lambda$ 95% ethanol-NaOH 293, $\lambda_{\text{max}}$ 321, 420 μ, log ε 4.07, 4.12, 3.89.

Anal. Calcd. for C$_{25}$H$_{26}$O$_5$: C, 73.87; H, 6.45; O, 19.68; Mol. Wt., 406. Found: C, 73.78; H, 6.60; O, 19.56; Mol Wt., 322.

The n.m.r. spectrum is reproduced on p. 120 (Figure 3). The relative integral is measured as follows:

-4.58 ½ (0.9); 2.72 ½ (5.2); 4.19 ½ (1); 7.10 and 7.13 ½ (total 3.8); 7.4-8.4 ½ (3.3); 8.53 ½ (6.3); 9.05 ½ (6.1).


3. (a) J. Polonsky and E. Lederer, Bull. Soc. Chim. France, 925 (1954); (b) J. Polonsky, ibid., 541 (1955); (c) ibid., 914 (1956); (d) ibid., 1079 (1957); (e) A. Ormancey-Potier, A. Buzas and E. Lederer, ibid., 577 (1951); (f) J. Polonsky, ibid., 929 (1958).


12. (a) N. S. Bhacca, L. F. Johnson and J. N. Shoolery, *Varian High Resolution Nuclear Magnetic Resonance Spectra Catalog*, Varian Associates, Palo Alto, California, 323; (b) *ibid.*, 139; (c) *ibid.*, 344; (d) *ibid.*, 366.


16. I thank Mr. B. A. Modi for the extraction.