taining approximately 1 mg. of active ingredient subjected to infrared analysis. As expected, the spectra of the tablets containing the physical mixture displayed absorption bands throughout the carbonyl region (5.67, 6.2, and 6.3 μ) typical of the individual components (Fig. 3a), while those of the tablets containing complexes displayed peaks characteristic of the pure complex (Fig. 3b). Thus, the presence of the tablet excipients did not interfere with this method of identification. The results also showed that complex formation did not occur during formulation of the tablet, nor during powdering or compression of the specimen in preparing the potassium bromide disk.

Four commercial products containing phenobarbital and quinidine or hydroquinidine alkaloids were subsequently examined by this method. Results obtained are shown in Fig. 3c–f. Only one of the preparations displayed absorption at 5.67 μ and lacked the intense absorption near 6.4 μ (Fig. 3d), indicating the presence of free phenobarbital and hence the presence of a mere physical mixture of the active ingredients. All other spectra (Fig. 3c,e,f) were characteristic of complexes.

By means of the experimental data presented, phenobarbital–quinine type complexes may be differentiated from physical mixtures of their components. This should prove of value in applying uniformity of legislation regarding the manufacture and sale of these pharmaceutical dosage forms.

REFERENCES

(1) Busquet, H., and Vischniac, C., *Compt. Rend. Soc. Biol.*, **119**, 503(1935).
(2) Mossini, A., and Recordati, G., *Boll. Chim. Farm.*, **74** 638(1935).
(3) Brit. pat. 674,807 (1952), Roche Products Ltd.
(4) Higuchi, T., and Lach, J. L., *J. Am. Pharm. Assoc. Sci. Ed.*, **43**, 349(1954).
(5) Arieson, V., *et al.*, *Farmacia*, **9**, 65(1961).
(6) Price, W. C., *et al.*, *J. Pharm. Pharmacol.*, **6**, 522(1954).
(7) Levi, L., and Hubley, C. E., *Anal. Chem.*, **28**, 1591 (1956).
(8) Williams, P. P., *ibid.*, **31**, 140(1959).
(9) Cleverley, B., and Williams, P. P., *Chem. Ind.*, **1959** 49.

Chemical Examination of a Toxic Extract of *Indigofera endecaphylla*

The Endecaphyllins

By R. A. FINNEGAN and W. H. MUELLER*

Chromatography on silica gel of a toxic acetone extract of leaves and stems of *Indigofera endecaphylla* Jacq. has provided, in addition to 3-nitropropanoic acid, ethyl 3-nitropropanoate, succinic acid, and methyl β-D-glucopyranoside, a series of nine 3-nitropropanoate esters of glucose. Six of these, endecaphyllins A, A_1, A_2, B, B_1, and C are isomeric triesters while three, C_1, D, and E, are diesters. A fourth nitro-containing glucose diester, endecaphyllin I, was also obtained by chromatography and is suggested to contain as an esterifying acid a nitro-acid other than 3-nitropropanoic. The eleventh member of this group, endecaphyllin X, was isolated directly from the crude extract and found to be a glucose tetra-(3-nitropropanoate) ester. Some chemical and physical properties of these compounds are described.

INTEREST in the chemical constituents of *Indigofera endecaphylla* Jacq. (creeping or trailing indigo), a tropical legume which had achieved status as a forage crop (2), stems from observations (3) of its production of acute toxic symptoms, frequently fatal, in cattle and other animals (4). Earlier chemical examination of this plant resulted in the isolation and identification of 3-nitropropanoic acid (5, 6) which previously had been shown (7) to be identical with hiptagenic acid, a hydrolysis product of hiptagin (8) and karakin (9–11). These latter two substances, along with 3-nitropropanoic acid and 2-phenyl-1-nitroethane (12), appear to be the only naturally occurring aliphatic nitro-compounds which have, until now, been reported (1, 13).[1]

Toxicity studies with *I. endecaphylla* extracts as well as with pure 3-nitropropanoic acid indicated that, while both showed high activity in chick feeding tests (5, 6, 14), the acid did not produce the severe liver damage in other animals which was found to be a characteristic effect of the

Received March 19, 1965, from the Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo.
Accepted for publication June 7, 1965.
Based on a thesis submitted by W. H. Mueller to the Department of Chemistry, The Ohio State University, Columbus, in partial fulfillment of Doctor of Philosophy degree requirements.
This investigation was supported by research grants GM-11412 and RG-8004 from the Division of General Medical Sciences, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
The authors acknowledge the technical assistance of Mrs. Ursula Mueller.
For a preliminary account of some of these results, see *Reference 1.*
* Present address: Esso Research and Engineering Co., Linden, N. J.

[1] A more complete dosier on naturally occurring nitro-compounds is given by Mueller, W. H., Thesis, The Ohio State University, Columbus, 1964. *Added in proof:* Just recently, Burrows, B. F., Mills, S. D., and Turner, W. B., *Chem. Comm.*, **1965**, 75, have observed 1-amino-2-nitrocyclopentane carboxylic acid to be a metabolite of *Aspergillus wentii.*

crude extract (15, 16). The conclusion that other toxic constituents must be present in the extract prompted the present investigation.

DISCUSSION

A crude toxic acetone extract of dried leaves and stems of *I. endecaphylla* was kindly supplied by Dr. Murrell P. Morris, Food and Drug Administration, Washington, D. C. Preliminary chromatographic studies on paper and with columns (cellulose powder, alumina, silica gel) showed that the most promising resolution of this extract occurred on silica gel. Accordingly, portions of solvent free extract were chromatographed on silica gel columns using chloroform and chloroform–ethanol mixtures as eluting solvents. The materials which were isolated in this manner are listed in Table I in the order of their elution from the columns.

Ethyl 3-Nitropropanoate.—A distillable oil, isolated early in the chromatography, was identified as ethyl 3-nitropropanoate by comparison of its infrared, ultraviolet, and NMR spectra, as well as its gas chromatographic retention time with those of an authentically prepared specimen. Since naturally occurring acids, although frequently encountered as methyl esters, have been found only rarely as ethyl esters (*Reference 17*, pp. 41 and 295), the authors considered the possibility that this ester was formed on the column during elution of the corresponding acid. In view of the fact that the ester was eluted before any ethanol had been added to the solvent used for elution (chloroform, water-saturated), the likelihood of esterification occurring on the column seems remote. It is conceivable, however, that ethanol was already present in the extract either as a plant constituent or as an impurity in the acetone and that the ester was formed by a transesterification reaction.[2]

3-Nitropropanoic Acid (Hiptagenic Acid).—The first crystalline compound to be eluted from the column was an acidic substance, m.p. 66.5–67.5°. This material was easily identified as the anticipated 3-nitropropanoic acid by comparison of its properties with those of a sample prepared by the method of Gresham and his co-workers (18). As mentioned before, this acid had been isolated previously from *I. endecaphylla.* It occurs in *Viola odorata* (19) and in *Corynocarpus laevigata* (20), and it is of interest that the compound is also a mold metabolite, being produced by cultures of *Aspergillus flavus* (21, 22), *A. oryzae* (23), and *Penicillium atrovenetum* (24). Attention has very recently been directed toward its mode of biosynthesis by the latter organism (25–28, 39).

Succinic Acid.—A second acidic crystalline substance was obtained during the course of chromatography and recognized as succinic acid from its melting point, infrared spectra, and microanalytical data. This assignment was verified by the customary comparisons with an authentic sample. The presence of this acid is unexceptional as it enjoys widespread occurrence in the plant kingdom (*Reference 17*, p. 237).

[2] It seems very unlikely that the ester was formed by direct reaction with the acid. A solution of 3-nitropropanoic acid in ethanol which was stirred for 12 days in the presence of suspended silica gel provided the ester in only 4% yield.

Methyl β-D-Glucopyranoside.—The last crystalline compound obtained from the column (see Table I) exhibited two melting points: 92–94° and 112–114°. The infrared spectrum (strong hydroxyl absorption) and microanalytical results, coupled with the observation of a positive Molisch test (29) and high water solubility, classified the substance as a carbohydrate. The presence of an *O*-methyl group was revealed by the NMR spectrum and confirmed by a Zeisel determination. The compound could be exhaustively methylated and acetylated, providing in each case a crystalline derivative. However, the microanalytical data which were available at this stage (parent and two derivatives) could not distinguish between a methyl hexose and a methyl pentose formulation for the parent substance. It was not until a benzylidene derivative was obtained and analyzed that data were obtained requiring a methyl hexose formula. With this recognition, the structural assignment followed quickly after comparison of the observed melting points and rotations with values reported in the literature for methyl β-D-glucopyranoside and its derivatives. Direct comparison (infrared spectrum, mixed melting point, and X-ray powder diffraction pattern) with an authentic sample of the glucoside left no doubt concerning its identity. Although this susbstance is a well known chemical (30) available commercially in bulk quantities, it is not generally known as a natural product, having been reported previously on only a few occasions (31).

The Endecaphyllins.—The general similarity in the properties of the endecaphyllins makes it convenient to discuss them as a group. They are white, crystalline compounds soluble in the more polar solvents such as acetone, dimethyl sulfoxide, acetonitrile, and tetrahydrofuran and are readily crystallized from acetone–chloroform and ethanol–chloroform mixtures. It should be pointed out that although some of their melting points are quite close or even identical (Table I), mixed melting points are strongly depressed, thus substantiating their individuality. The infrared spectra (potassium bromide) exhibited many features in common. All show hydroxyl absorption near 3500 $cm.^{-1}$, bands in the regions near 1735 and 1180 $cm.^{-1}$ indicating the presence of saturated ester groupings, and bands near 1550 $cm.^{-1}$ characteristic of the nitro function. In addition, all show a pronounced band near 870 $cm.^{-1}$ which may be ascribed to the bending vibration of the anomeric C—H bond (32) and taken as indication of the β configuration of the substituent at that center. The presence in these molecules of nitro groups as suggested by the infrared spectra, and in particular, primary aliphatic nitro groupings, $—CH_2NO_2$, was verified by the observation of positive red reactions in the red, white, and blue tests (33) which were carried out using the procedure of Dannley and Kitko (34). The conclusion that all the nitrogen atoms were present in nitro groups was supported by the ultraviolet spectra of the endecaphyllins which showed a single absorption band near 275 mμ. These data are listed in Table II, where it may be seen that the size of the extinction coefficients is in excellent accord with that expected (35) for the coincidence of nitro groups with nitrogen atoms.

Table II contains, in addition, the results of quantitative microhydrogenation experiments which

TABLE I.—COMPOUNDS ISOLATED FROM *I. endecaphylla*

Compd.	M.p. (b.p.), °C.	Formula	% Yield[a]
Ethyl 3-nitropropanoate	b.p. 67°/1 mm.	$C_5H_9NO_4$	1.1
3-Nitropropanoic acid	66.5–67.5	$C_3H_5NO_4$	5.6
Endecaphyllin I	139–140	$C_{16}H_{26}N_2O_{12}$	0.1
Endecaphyllin A	120–122	$C_{15}H_{21}N_3O_{15}$	1.4
Endecaphyllin A_1	81–91	$C_{15}H_{21}N_3O_{15}$	0.7
Endecaphyllin A_2	156–158.5	$C_{15}H_{21}N_3O_{15}$	0.05
Succinic acid	184–186	$C_4H_6O_4$	0.3
Endecaphyllin B	125–126.5	$C_{15}H_{21}N_3O_{15}$	2.1
Endecaphyllin B_1	129–130	$C_{15}H_{21}N_3O_{15}$	0.7
Endecaphyllin C	155–156	$C_{15}H_{21}N_3O_{15}$	4.7
Endecaphyllin C_1	145–146.5	$C_{12}H_{18}N_2O_{12}$	0.8
Endecaphyllin D	145–146	$C_{12}H_{18}N_2O_{12}$	2.2
Endecaphyllin E	132–134	$C_{12}H_{18}N_2O_{12}$	1.2
Endecaphyllin X[b]	104–105.5	$C_{18}H_{24}N_4O_{18}$	...
Methyl β-D-glucopyranoside	112–114	$C_7H_{14}O_6$	1.1

[a] These figures represent the percentage of purified compound based on the amount of solvent free extract chromatographed. Multiplication by a factor of 10^{-2} gives the per cent yield based on dried plant material. [b] This compound was isolated directly from the extract without chromatography and supplied in crude form by Dr. M. P. Morris. It has not so far been obtained by the separation methods described here.

TABLE II.—ULTRAVIOLET, HYDROGENATION, AND SAPONIFICATION DATA FOR THE ENDECAPHYLLINS

Compd.	N Atoms, No.	$\lambda_{max.}^{95\%\ ethanol}$ mμ	ε	ε/N Atom	Hydrogen Uptake, *M* Equiv. Calcd.[a]	Found	Alkali Consumption, *M* Equiv. Calcd.[b]	Found
3-Nitropropanoic acid	1	273	23.7	23.7	3.0	3.0	2.0	1.90
	...	...	...	...	...	...	...	1.95
Ethyl 3-nitropropanoate	1	270	21.2	21.2	3.0	3.0	2.0	1.73
	...	...	...	...	...	...	...	1.79
Endecaphyllin								
I	2	276	39.9	19.9	6.0	[c]	4.0	[c]
A	3	272	73.0	24.3	9.0	9.3	6.0	6.02
..	...	...	...	...	...	8.3[d]	...	5.86
A_1	3	273	74.6	24.9	9.0	[c]	6.0	6.16
A_2	3	[c]			9.0	8.5	6.0	[c]
B	3	270	106.8	34.3	9.0	8.5	6.0	6.28
..	...	...	...	...	...	8.9	...	6.06
B_1	3	275	75.5	25.2	9.0	9.2	6.0	5.96
..	...	...	...	...	...	...	...	6.18
C	3	277	75.7	25.2	9.0	9.2	6.0	5.95
..	...	...	...	...	...	9.1	...	5.82
C_1	2	274	56.5	28.2	6.0	6.1[d]	4.0	4.08
..	...	...	...	...	...	...	...	3.99
D	2	273	51.0	25.5	6.0	5.7[d]	4.0	3.70
..	...	...	...	...	...	5.7	...	3.91
E	2	276	37.0	18.5	6.0	6.3	4.0	4.08
..	...	...	...	...	...	5.8	...	4.02
X	4	274	99.0	24.8	12.0	12.7[d]	8.0	8.03
..	...	...	...	...	...	12.4[d]	...	8.08
..	...	...	...	...	...	10.8[d]	...	...

[a] Calculated for the uptake of 3 moles of hydrogen per nitro group. [b] Calculated for the consumption of one equivalent per ester group plus one per nitro group. [c] Insufficient material was available for this measurement. [d] In all the hydrogenation experiments, the uptake of gas was plotted against time. These values are inflection points on the curve where the rate of uptake slackened considerably. In these cases, the final uptake values were markedly higher than the calculated ones.

provided hydrogen uptake values consistent with the complete reduction of all nitro groups to amino groups, *i.e.*, $R—NO_2 + 3\ H_2 \rightarrow R—NH_2 + 2\ H_2O$. Initial attempts to carry out these reductions using a variety of catalysts (palladium, platinum, ruthenium on charcoal, platinum oxide) and solvents (ethanol, acetic acid, dimethylformamide) met with failure, since hydrogen uptake either did not occur at all, or else proceeded at such a slow pace that the results were erratic and irreproducible. Carter (11) has already noted the resistance to catalytic hydrogenation offered by karakin (*vide infra*) and 3-nitropropanoic acid. Acceptable results were obtained, however, following the discovery that a trace of base added to the hydrogenation solvent markedly accelerated the hydrogen uptake. The data listed in Table II were obtained using platinized charcoal as catalyst in ethanol containing 0.05% triethylamine as solvent. That the rate of reduction is enhanced in the presence of added base may be an indication that the aci-nitro form of the substrate is involved.

Finally, Table II contains one additional set of quantitative information, the saponification equivalents. It was concluded that under the conditions of these experiments, an equivalent of alkali was consumed not only in the hydrolysis of ester groups, but also by the formation of aci-nitro salts. In each case, the data are consistent with the presence of one nitro function *per* ester grouping.

To the data set forth above may now be added the results obtained by acid hydrolysis of the endecaphyllins followed by paper chromatography of the hydrolysates. In each case glucose was de-

tected; and, in all but four instances, 3-nitropropanoic acid was also detected as a hydrolysis product.[3] These observations, when taken with those already described, lead to the formulation of endecaphyllins C_1, D, and E as isomeric di-*O*-(3-nitropropanoyl)-D-glucopyranoses; of compounds A, A_1, A_2, B, B_1, and C as isomeric tri-esters, and of compound X as a tetra-ester. Some additional experiments which pertain to certain individual members of this group of compounds are discussed in the sections which follow.

Endecaphyllin I.—This material does not conform to the simple pattern of nitropropanoyl esters of glucose outlined above. On the basis of its molecular formula (Table I) and its general spectral similarity to the other endecaphyllins, it seemed reasonable to suppose that it was a glucose di-nitropropanoate ester containing, in addition, four carbons arranged in one or more ether functions, *i.e.*, $C_6H_9O_6(COCH_2CH_2NO_2)_2(C_4H_9)$. However, the fact that glucose (and not a glucose ether) was detected after both acid and base catalyzed hydrolysis indicated that the C_4 residue must be a part of the acyl portion of the ester. Although 3-nitropropanoic acid was not detected[3] in these hydrolysates, a component was present which was common to all the endecaphyllins, possibly an isomeric mono-ester of glucose with 3-nitropropanoic acid. The limited data available suggest that endecaphyllin I consists of a glucose moiety which is esterified by one molecule of 3-nitropropanoic acid and by 1 molecule of a new nitro acid with the formula $O_2N(C_6H_{12})CO_2H$. The acquisition of an additional quantity of this substance will undoubtedly lead to clarification of this issue.

Endecaphyllin A.—The physical properties (m.p. 120–122°; $[\alpha]_D^{22}$, 4.3°) and molecular formula of endecaphyllin A suggest that it is identical with karakin (m.p. 120°; $[\alpha]_D^{20}$, 4.5°) for which the structure 1,4,6-tri-*O*-(3-nitropropanoyl)-β-D-glucopyranoside has been advanced (11). Unfortunately, the authors have been unable to verify this identification as the conditions reported by Carter (11) to provide karakin diacetate, m.p. 103°, produced a mono-acetate, m.p. 124.5–126.5°, when applied to endecaphyllin A. A direct comparison of karakin and endecaphyllin A would be desirable, and to this end the reisolation of karakin is presently being attempted.

Endecaphyllin B_1.—The original formulation (1) of endecaphyllin B_1 as a tri-ester of a methyl glucoside is now revised to a tri-ester of glucose. New microanalytical data are in better accord with the latter formulation and furthermore, both acid and basic hydrolysis leads to glucose rather than to a glucose methyl ether.

Endecaphyllin C.—A classical approach to the determination of the structure of the endecaphyllins would involve exhaustive methylation followed by saponification and identification of the resulting glucose methyl ethers. Accordingly, an attempt was made to apply this general method to endecaphyllin C, the most abundant of this group of compounds (Table I). Since the application of Kuhn's methylation procedure (31) to endecaphyllin C furnished only tarry products,[4] the authors turned to the acid catalyzed diazomethylation technique of Neeman (38).[5] The stability of the glucose nitroester moiety under the conditions of this reaction (diazomethane in methylene chloride solution with boron trifluoride as catalyst) was assured when a synthetic sample of methyl tetra-*O*-(3-nitropropanoyl)-β-D-glucopyranoside, m.p. 109–110°, was found to be unaffected and could be recovered quantitatively from such a reaction mixture. Additional encouragement was provided by the observation that a synthetic methyl tri-*O*-(3-nitropropanoyl)-β-D-glucopyranoside, m.p. 147–148°, was converted in good yield to a methylated derivative, m.p. 126–127.5°, by treatment of a methylene chloride suspension of the glucoside with a sixteenfold excess of diazomethane and a catalytic amount of boron trifluoride.[6] Application of the method to endecaphyllin C, however, gave disappointing results. Repeated attempts using a thirty- to forty-fold excess of diazomethane afforded only about 30% of a mixture of methylated material. This mixture separated on silica gel thin layers into three components (R_f 0.70, 0.75, 0.93, see *Experimental*), and remethylation did not improve the product distribution. Furthermore, crystalline products could not be obtained by column chromatography. Although previous studies (38) had indicated that methylene chloride was a solvent of choice for this reaction, additional experiments were carried out using tetrahydrofuran as solvent with the hope that a homogeneous reaction mixture would be beneficial. In order to combat the potentially deleterious interaction of the solvent with the catalyst, larger amounts of catalyst were employed. Although only 10% of starting material was recovered, the methylation product was again a mixture containing the components with R_f 0.75 and 0.93. After several crystallizations of this mixture, a pure compound, m.p. 98–101°, R_f 0.75, was eventually obtained. The microanalytical data and saponification equivalent obtained for this substance classified it as a di-*O*-(3-nitropropanoyl)-*O*-methyl-D-glucose derivative, indicating that, while a methyl group had been introduced, a nitropropanoyl residue had been removed. The presence of a glucose methyl ether in the acid hydrolysate was demonstrated by thin-layer chromatography. Since the methyl group survived the acid hydrolysis, it cannot be located at C_1; however, the missing nitro-ester group very likely had been located at C_1 in view of the stability, mentioned above, of the synthetic tetra-*O*-(3-nitropropanoyl)-β-methyl-D-glucopyranoside under the same reaction conditions. The component with R_f 0.93 probably stems from further methylation of the di-ester, while the one with R_f 0.70 may very well be the desired di-*O*-methyl-tri-*O*-(3-nitroprop-

[3] Since the acid itself provides only a very faint spot in these analyses, failure to detect it may not be taken as indication of its absence. Indeed, in one case where it was not detected in the acid hydrolysate (endecaphyllin A), it was isolated in crystalline form after hydrolysis of the starting material in boiling water; and furthermore, it could be detected on paper after partial hydrolysis under these conditions. In another case where it was not detected (endecaphyllin A_2), an identical substance has been synthesized by the acylation of glucose with 3-nitropropanoyl chloride (36).

[4] This sensitivity of the endecaphyllins to base is perhaps not surprising in view of the potential reactivity of the nitro-bearing methylene groups in aldol condensations as well as the (presumed) susceptibility of the nitro groups to β-elimination. Tar formation was also observed during synthetic experiments when pyridine was used as solvent (36).

[5] The authors thank Dr. Neeman for helpful discussions regarding this method.

[6] These synthetic materials will be described in a subsequent article. See *Reference 36.*

anoyl)-D-glucose derivative since R_f 0.69 was observed for a synthetic methyl *O*-methyl-tri-*O*-(3-nitropropanoyl)-β-D-glucoside (m.p. 126–127.5°; see above). The limited amounts of starting materials available at this time have precluded further experimentation along these lines.

The detailed structures of the endecaphyllins are expected to result from synthetic and degradative studies now in progress.[7] In addition, the authors hope to provide further information concerning the biological activity of various members of this class of compounds.

EXPERIMENTAL

The infrared spectra were measured on the Perkin-Elmer models number 137B, 21, and 237 spectrophotometers. A Cary model 14 and a Perkin-Elmer model 202 were used to record the ultraviolet spectra. A Varian Associates A 60 spectrometer was used for the NMR spectra. Microanalyses and molecular weight determinations (Rast) were performed by Dr. A. Bernhardt, Mülheim, Germany. Unless otherwise noted, the melting points were observed on a Fisher-Johns 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.).

Isolation

A dark brown acetone extract (about 400 Gm. 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 Gm. of solvent free material was chromatographed on 650 Gm. of silica gel, packed in skellysolve B. The column used was 700 mm. long and 45 mm. in diameter. With a flow rate of 100 ml./hr., 500 1-ml. fractions were collected. Skellysolve B was slowly replaced by water-saturated chloroform.

3-Nitropropanoic Acid.—Upon eluting with (water-saturated) chloroform, the first crystalline compound was obtained (fraction No. 27–41). The crude yellow material, m.p. 62.5–66°, was recrystallized 3 times from chloroform affording 300 mg. (5.6% yield) of white plates, m.p. 65.5–67.5°. $\nu_{max.}^{KBr}$ 3100, 1715, 1560 cm.$^{-1}$; $\lambda_{max.}^{95\% \text{ ethanol}}$ 273 mμ, e 23.7. The NMR 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 $C_3H_5NO_4$: C, 30.26; H, 4.37; N, 11.76; O, 53.75; mol. wt., 119. Found: C, 30.52; H, 4.37; N, 12.00; O, 52.07; mol. wt., 132.

The mixed melting point with an authentic sample (18) of 3-nitropropanoic acid was undepressed (66.5–67.5°), and the infrared spectra of the two samples were indistinguishable.

Continuation of the chromatography with small increases of ethanol to (water-saturated) chloroform furnished the endecaphyllins.

[7] Also planned are experiments designed to assess the possibility that one or more of the endecaphyllins was formed by disproportionation or rearrangement reactions during the isolation.

Endecaphyllin A.—With 2% ethanol in (water-saturated) chloroform (fractions No. 76–84) tan crystals, melting at 109–117°, were obtained. Four recrystallizations from an acetone–chloroform mixture gave 77 mg. (1.4% yield) of white crystals, m.p. 120–122°; $\nu_{max.}^{KBr}$ 3510, 1750, 1725 (sh), 1555 (split), 1372, 1170, 1093, 868 cm.$^{-1}$; $\lambda_{max.}^{95\% \text{ ethanol}}$ 272 mμ, e 73.4; $[\alpha]_D^{22}$ = 4.3° (5% in acetone, 1 dm.).

Anal.—Calcd. for $C_{15}H_{21}N_3O_{15}$: C, 37.30; H, 4.38; N, 8.70; O, 49.69; mol. wt., 483. Found: C, 37.19, 36.98; H, 4.20, 4.25; N, 8.82, 8.87; O, 49.73, 48.82; mol. wt., 436.

Succinic Acid.—A semisolid material (fractions No. 93–105) was eluted with 3% ethanol in water-saturated chloroform. When crystallization attempts failed, the mixture was rechromatographed on silica gel, which yielded crystalline succinic acid with 3% ethanol in water-saturated chloroform as eluent. The crude material melting at 150–184° was recrystallized 3 times from an acetone–hexane mixture which afforded 16 mg. (0.3% yield) of white crystals, m.p. 184–186° (sealed tube, sublimes). $\nu_{max.}^{KBr}$3100 (broad), 1755 (sh), 1715 cm.$^{-1}$.

Anal.—Calcd. for $C_4H_6O_4$: 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 produced no depression of the melting point (183–185° sealed tube, sublimes), and the infrared spectra of the isolated material was indistinguishable from that of the authentic sample.

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 an acetone–chloroform mixture, which raised the melting range to 81–91°. Further recrystallizations did not result in sharp melting material. The 35 mg. (0.7% yield) of white crystals appeared to be uniform (R_f 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 $\nu_{max.}^{KBr}$ 3340 (split), 1745, 1555, 1370, 1177, 1035, 868 cm.$^{-1}$; $\lambda_{max.}^{ethanol}$ 273 mμ, e 74.5.

Anal.—Calcd. for $C_{15}H_{21}N_3O_{15}$: C, 37.30; H, 4.38; N, 8.70; O, 49.69; mol. wt., 483. Found: C, 37.69; H, 4.72; N, 8.62, 8.26; O, 49.47; mol. wt., 379, 357.

Endecaphyllin B.—Endecaphyllin B was obtained with the same solvent mixture as was succinic acid—3% ethanol in water-saturated chloroform (fractions No. 106–111). The isolated tan material, m.p. 112–119°, was recrystallized from an acetone–chloroform mixture, then 2 times from ethanol yielding 115 mg. (2.1% yield) of white needles melting at 125–126.5°. $\nu_{max.}^{KBr}$ 3530, 1765 (sh), 1738, 1555, 1400, 1255, 1179, 1087, 872 cm.$^{-1}$; $\lambda_{max.}^{95\% \text{ ethanol}}$ 279 mμ, e 106.

Anal.—Calcd. for $C_{15}H_{21}N_3O_{15}$: C, 37.30; H, 4.38; N, 8.70; O, 48.69; mol. wt., 483. Found: C, 37.36, 37.47; H, 4.72, 4.56; N, 8.53, 8.47; O, 49.26, 49.01; mol. wt., 458, 352.

Endecaphyllin C.—Water-saturated chloroform with 3.0–4.5% ethanol furnished endecaphyllin C as tan crystals (fractions No. 117–137). The iso-

lated crude material melted at 135–148° (dec.). Five recrystallizations from an acetone–chloroform mixture afforded 254 mg. (4.7% yield) of a white crystalline compound, m.p. 155–156° (very little decomposition). $\nu_{max.}^{KBr}$ 3600, 1745, 1551, 1385, 1200, 1025, 875 cm.$^{-1}$; $\lambda_{max.}^{95\%\ ethanol}$ 270 mμ, e 75.7.

Anal.—Calcd. for $C_{15}H_{21}N_3O_{15}$: C, 37.30; H, 4.38; N, 8.70; O, 49.69; mol. wt., 483. Found: C, 37.38, 37.39; H, 4.50, 4.42; N, 8.67, 8.52; O, 48.42, 47.42; mol. wt., 407, 394.

Endecaphyllin D.—With 6.5% ethanol in water-saturated chloroform endecaphyllin D was eluted (fractions No. 160–173). The crude material was tan crystals with m.p. 134–141°. Two recrystallizations from an acetone–chloroform mixture afforded 120 mg. (2.2% yield) of white crystals, m.p. 145–146°. $\nu_{max.}^{KBr}$ 3500, 1733 (split), 1552, 1390, 1195, 1030, 873 cm.$^{-1}$; $\lambda_{max.}^{95\%\ ethanol}$ 271 mμ, e 51.

Anal.—Calcd. for $C_{12}H_{18}N_2O_{12}$: C, 37.73; H, 4.75; N, 7.33; O, 50.26; mol. wt., 382. Found: C, 37.96, 38.48; H, 4.83, 4.92; N, 7.35, 6.94; O, 48.75; mol. wt., 369.

Endecaphyllin E.—With water-saturated chloroform, containing 7.0% ethanol, endecaphyllin E was obtained (fractions No. 179–191). The isolated tan material, m.p. 122–127°, was recrystallized 3 times from an acetone–chloroform mixture yielding 65 mg. (1.2% yield) of white crystals, m.p. 132–134°. $\nu_{max.}^{KBr}$ 3450, 1736, 1550, 1392, 1190, 1024, 869 cm.$^{-1}$; $\lambda_{max.}^{95\%\ ethanol}$ 276 mμ, e 37.

Anal.—Calcd. for $C_{12}H_{18}N_2O_{12}$: C, 37.73; H, 4.75; N, 7.33; O, 50.26; mol. wt., 382. Found: C, 37.43; H, 5.09; N, 7.81; O, 49.52; mol. wt., 357.

Methyl β-D-Glucopyranoside.—With 20% ethanol in water-saturated chloroform methyl β-D-glucoside was isolated (fractions No. 252–266). The naturally occurring glucoside crystallized from ethanol in white plates. It exists in two dimorphic forms, m.p. 92–94° and 112–114°. [Reported (30) m.p. 108°; $[\alpha]_D^{22}$ = −29° (1% in water, 1 dm.); reported (30) −32°.] The infrared spectrum and X-ray powder diffraction pattern are[8] indistinguishable from those of an authentic sample, and a mixed melting point with an authentic sample (m.p. 114–115°) showed no depression, m.p. 112–115°. A NMR spectrum was taken in D_2O (about 15% concentration), which shows a doublet at 5.76 τ (J = 7.4 c.p.s.), and a singlet at 6.61 τ. All additional signals are not well resolved. Dioxane was used as internal reference standard (τ = 6.43).

Anal.—Calcd. for $C_7H_{14}O_6 \cdot {}^1/_2\ H_2O$: C, 41.41; H, 7.45; O, 51.23; CH_3O, 15.26. Found: C, 41.51; H, 7.44; O, 50.79; CH_3O, 15.60.

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 $C_7H_{14}O_6 \cdot {}^1/_2\ H_2O$.

Anal.—Found: C, 41.68; H, 7.32; O, 50.80.

X-ray powder diffraction patterns[8] of the isolated and the sublimed samples were identical. Either the glucoside never lost its crystal water or picks it up instantly when exposed to moist air.

In several column chromatography attempts on a larger scale, ethyl-3-nitropropanoate and four additional endecaphyllins were isolated.

[8] The authors are grateful to Professor Sheldon Shore, The Ohio State University, for these measurements.

Ethyl 3-Nitropropanoate.—When 28.4 Gm. of solvent-free extract was chromatographed on 1750 Gm. of silica gel in the same manner as described before, 300 mg. (1.1% yield) of a yellow liquid was isolated using water-saturated chloroform as eluent (fractions No. 35–44). 3-Nitropropanoic acid came off the column 44 fractions later, while still using the same solvent. Distillation provided a colorless liquid, b.p. 40–50°/0.04 mm. (bulb to bulb). $\nu_{max.}^{film}$ 1740, 1555 cm.$^{-1}$; $\lambda_{max.}^{95\%\ ethanol}$ 270 mμ, e 21.2. A synthetic sample showed b.p. 55°/0.25 mm. ν_{max}^{film} 1740, 1555 cm.$^{-1}$; $\lambda_{max.}^{95\%\ ethanol}$ 270 mμ, e 21.1. The infrared spectrum of the naturally occurring ester is very similar to that of a synthetic sample prepared by Fischer esterification of the corresponding acid. Gas chromatographic analysis (113°, 75 ml./min., 20% SF-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 NMR 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 $C_5H_9NO_4$: C, 40.81; H, 6.17; O, 43.50; mol. wt., 147. Found: C, 41.12; H, H, 6.23; O, 43.28; mol. wt., 157.

A solution of 500 mg. 3-nitropropanoic acid in 15 ml. of ethanol (absolute) was stirred for 12 days at room temperature in the presence of 5 Gm. of silica gel. The silica gel was then removed by filtration and the solvent evaporated 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 3-nitropropanoate was obtained after removal of the ether, and was identified by its infrared spectrum.

Endecaphyllin I.—Chromatography of 35.5 Gm. of solvent free extract on 2000 Gm. of silica gel yielded crude endecaphyllin I, m.p. 128–135°, with 2% ethanol in water-saturated chloroform (fractions No. 212–217). This was the first endecaphyllin obtained after the elution of ethyl 3-nitropropanoate and 3-nitropropanoic acid. After six recrystallizations from an acetone–chloroform–hexane mixture, 35 mg. (0.1% yield) of white crystals was obtained, m.p. 139–140.5°. $\nu_{max.}^{KBr}$ 3571, 1745, 1710, 1575, 1553 (sh), 1391, 1198, 877 cm.$^{-1}$; $\lambda_{max.}^{95\%\ ethanol}$ 276 mμ, e 39.8.

Anal.—Calcd. for $C_{16}H_{26}N_2O_{12}$: C, 43.87; H, 6.00; N, 6.40; O, 43.84; mol. wt., 438. Found: C, 43.93, 43.75; H, 5.68, 5.49; N, 6.05, 6.95; O, 43.44, 44.45; mol. wt., 386.

Endecaphyllin A$_2$.—From 14.2 Gm. of solvent free extract, chromatographed on 900 Gm. of silica gel, endecaphyllin A_2, m.p. 150–155°, was eluted as tan crystals with 2.5% ethanol in water-saturated chloroform (fractions No. 203–212). The compound was eluted after endecaphyllin A and before endecaphyllin B was obtained. Six recrystallizations from an acetone–ethanol–chloroform mixture afforded 7 mg. (0.05% yield) of white crystals, m.p. 156.5–158.5°. $\nu_{max.}^{KBr}$ 3490 (split), 1725 (split), 1550, 1385 (split), 1195 (split), 1045 (split), 870 cm.$^{-1}$. A mixed melting point with

endecaphyllin C (m.p. 155–156°) was depressed, m.p. 137–140°.

Anal.—Calcd. for $C_{15}H_{21}N_3O_{15}$: C, 37.30; H, 4.38; N, 8.70. Found: C, 37.38; H, 4.20; N, 8.88.

Endecaphyllin B₁.—This compound was isolated on the same column as endecaphyllin A_2 (fractions No. 234–249, 2.5% ethanol in water-saturated chloroform). Its elution began one fraction after endecaphyllin B and ended 35 fractions before endecaphyllin C (3% ethanol in water-saturated chloroform) was obtained. When recrystallized 3 times from an acetone–chloroform mixture, 95 mg. (0.7% yield) of white crystals was obtained, m.p. 129–130°. $\nu_{max.}^{KBr}$ 3560, 3310 (broad), 1763 (sh), 1740, 1558, 1370, 1272, 1093, 870 cm.$^{-1}$; $\lambda_{max.}^{95\% \text{ ethanol}}$ 275 mμ, e 75.5.

Anal.—Calcd. for $C_{15}H_{21}N_3O_{15}$: C, 37.30; H, 4.38; *N, 8.70; O, 49.69; mol. wt., 483. Found:* C, 37.68, 38.54; H, 4.43, 4.45; N, 8.49; O, 48.62, 47.33; mol. wt., 473.

Endecaphyllin C₁.—On 800 Gm. of silica gel 10.6 Gm. of solvent free extract was chromatographed. With 3% ethanol in water-saturated chloroform (fractions No. 332–341) tan, crystalline endecaphyllin C_1, m.p. 140–145°, was obtained immediately after the elution of endecaphyllin C. Three recrystallizations from ethanol afforded 73 mg. (0.8% yield) of white crystals, m.p. 145–146.5°. $\nu_{max.}^{KBr}$ 3450, 1745, 1715 (sh), 1555, 1382 (split), 1182, 1015, 870 cm.$^{-1}$. $\lambda_{max.}^{95\% \text{ ethanol}}$ 274 mμ, e 56.5. A mixed melting point with endecaphyllin D (m.p. 145–146°) was depressed, m.p. 126–137°.

Anal.—Calcd. for $C_{12}H_{18}N_2O_{12}$: C, 37.73; H, 4.75; 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 as a brown solid, m.p. 96–100°. Treatment with charcoal in acetone, and three recrystallizations from an acetone–hexane mixture afforded white crystals, m.p. 104–105.5°. $\nu_{max.}^{KBr}$ 3495 (broad), 1748, 1555, 1375, 1175, 1090, 872 cm.$^{-1}$; $\lambda_{max.}^{95\% \text{ ethanol}}$ 274 mμ, e 99.

Anal.—Calcd. for $C_{18}H_{24}N_4O_{18}$: C, 36.99; H, 4.14; N, 9.59; O, 49.28; mol. wt., 584. Found: C, 36.72, 39.12; H, 4.40, 4.40; N, 9.11; O, 47.54; mol. wt., 576.

Characterization

Hydrogenation.—The microhydrogenations were carried out on 3–5 mg. of compound in a thermostated room at approximately 10° and at atmospheric pressure. In the calculation of the hydrogen uptake, the vapor pressure of the solvent, atmospheric pressure, and temperature were accounted for. All reductions were carried out in 3 ml. of ethanol (absolute) containing 0.05% (v/v) triethylamine and 10 mg. of 5% Pt on carbon as a catalyst. The results are listed in Table II.

Color Test for Primary Nitro Groups (34).—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. of carbon tetrachloride were added. The mixture was slowly neutralized with 10% sulfuric acid. At pH 7–9, a yellow-brown color appeared indicating the presence of a $-CH_2NO_2$ grouping (a blue-green color indicates R_2CHNO_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. of glacial acetic acid and 1 ml. of 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. of water. An oily emulsion was formed from which the oil separated after standing for 3 hr. in the refrigerator. The aqueous phase was decanted, the oil dissolved in methanol containing 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 an acetone–methanol mixture, 41 mg. of white needles, m.p. 124.5–126.5°. $\nu_{max.}^{KBr}$ 3350, 1748, 1555, 1371, 1169, 1080, 869 cm.$^{-1}$.

Anal.—Calcd. for $C_{17}H_{23}N_3O_{16}$: C, 38.89; H, 4.42; O, 48.76; mol. wt., 525. Found: C, 38.57; H, 4.47; O, 48.10; 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 subjected to these conditions, no diacetate was obtained. No pure product was obtained when the acetylation was attempted using acetic anhydride in pyridine.

Partial 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. 109–110°. A mixed melting point with endecaphyllin A (m.p. 120–122°) was 110–120°. Its infrared spectrum is indistinguishable with that of endecaphyllin A. The mother liquor, a yellow oil, yielded from an acetone–chloroform mixture a few crystals of 3-nitropropanoic 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 a few crystals from an ethanol–water mixture, 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 R_f 0.65 for endecaphyllin A, R_f 0.69 for the compound with m.p. 101–104°, and R_f 0.74 for the mother liquor. A sample of 3-nitropropanoic acid showed R_f 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 hr. After 5–10 min., the compounds went into solution. When the hydrochloric acid was removed by a nitrogen jet,

Table III.—Paper Chromatographic Analysis of the Endecaphyllin Hydrolysates

Endecaphyllin	R_f Values						
A	0.05;	0.18;	...	0.32;	...	0.92;[a]	0.97[a]
B	0.05;	0.18;	0.24;	0.32;	0.78;	...	...
C	0.05;	0.18;	0.24;	0.38;	0.78;	...	...
D	0.05;	0.18;	0.24;	0.33;	0.78;	...	...
E	0.05;	0.18;	...	0.32;	0.78;	...	...
Dextrose	...	0.18;	...	...	...	...	...
3-Nitropropanoic acid	...	...	...	...	0.78;	...	...
I	0.03;	0.17;	...	0.32;	...	...	...
A_1	0.03;	0.17;	...	0.32;	0.77;	...	...
A_2	0.03;	0.17;	...	0.32;	...	...	...
B_1	0.03;	0.17;	...	...	...	...	...
C_1	0.03;	0.17;	0.24;	0.33;	0.78;	...	...
X	0.03;	0.17;	...	0.34;	0.78;	...	...
Dextrose	...	0.17;	...	...	...	...	...

[a] These spots were only recognized by their fluoresence under the ultraviolet lamp.

the remaining brown residue was dissolved in a few drops of an ethanol–water mixture. 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 *N* 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 R_f values in Table III were calculated for the hydrolysis products. The spots for the 3-nitropropanoic 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 R_f 0.18 (glucose) and R_f 0.32 were the largest and most intensive.

Base Hydrolysis of Endecaphyllin B_1.—A 1-mg. sample of endecaphyllin B_1 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 concentrated sulfuric acid, and subsequent heating for 15 min. at 110°. The observed R_f value of 0.18 was identical with the one for dextrose.

Similar treatment of a synthetic sample of methyl-tetra-*O*-(3-nitropropanoyl)-β-D-glucoside[6] furnished a spot identical with one obtained from methyl-β-D-glucoside (R_f 0.27).

Base Hydrolysis of Endecaphyllin I.—When endecaphyllin I was treated and chromatographed, as described above for endecaphyllin B_1, glucose was detected (R_f 0.17).

Saponification Equivalents of the Endecaphyllins.—Approximately 5 mg. of each of the endecaphyllins was dissolved in 1 ml. of acetone and 1 ml. of 0.1 *N* sodium hydroxide added. The solutions turned yellow instantly. After 30 min., the reaction mixtures were titrated with 0.01 *N* sulfuric acid, and the results are listed in Table II.

Methylation of Endecaphyllin C with Methyl Iodide.—According to the method of Kuhn (37), 50 mg. of endecaphyllin C was dissolved in 3 ml. of 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 hr. at 0° and for an additional 20 hr. at room temperature. During the period of stirring the mixture turned brown gradually. Twenty milliliters of chloroform was then added and the barium salts removed by filtration. The chloroform solution was washed 2 times with 10 ml. of water, once with 10 ml. of 10% aqueous sodium thiosulfate, and 4 additional times with a total of 40 ml. of water, dried over magnesium sulfate, and the solvent removed *in vacuo*. A black tar (26 mg.) was obtained.

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

Methylation of Endecaphyllin C in Methylene Chloride Solution.—A 100-mg. sample of endecaphyllin C (0.205 mmole) was suspended in 5 ml. of dry methylene chloride. After the addition of 0.1 ml. of boron trifluoride catalyst stock solution (prepared by adding 0.114 ml. freshly distilled boron trifluoride-etherate to 10 ml. dry methylene chloride), 20 ml. of a 0.66 *M* diazomethane solution in methylene chloride (13.2 mmoles) was 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. of methylene chloride, and the insoluble material together with the formed polymethylene was removed by filtration. The filtrate was washed several times with a total of 15 ml. of water, dried over $MgSO_4$, and the solvent evaporated *in vacuo* to give 30.4 mg. of a faint yellow oil. 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 concentrated H_2SO_4, followed by heating for 10 min. at 110°, showed three components (R_f 0.70, 0.75, and 0.93). Endecaphyllin C, spotted on the same plate had R_f 0.76. However, the component with R_f 0.75 present in the yellow oil is not identical with endecaphyllin C, since they could be distinguished

by spotting them on paper. Attempts to crystallize this oil failed. Column chromatography on 1 Gm. of silica gel with isohexane–chloroform and chloroform–ethanol mixtures as eluent did not yield any crystalline fractions. The oily fractions obtained still showed similar mixtures on thin layers.

The solid obtained by filtration of the reaction mixture (above) was heated in 5 ml. of acetone, and the acetone insoluble polymethylene removed by filtration. On evaporating the filtrate, a colorless oil was obtained, which afforded on crystallization from an acetone–chloroform mixture 67.5 mg. of white crystals, m.p. 135–149°. Its infrared spectrum was 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. of 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 R_f 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 *M* 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 *M* 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 R_f values on thin-layer chromatography (R_f 0.70, 0.76, and 0.93). The recovery of starting material was 31 mg.

Methylation of Endecaphyllin C in Tetrahydrofuran.—A sample of 50 mg. of endecaphyllin C was dissolved in 1.5 ml. tetrahydrofuran (freshly distilled from lithium aluminum 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. Its infrared spectrum and the abnormal weight increase suggested the presence of a by-product derived from the solvent.

Remethylation of the starting material (31 mg.) 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 crystallization from an acetone–chloroform–hexane mixture yielded 54 mg. of a gummy solid, which afforded, after two further recrystallizations, 25 mg. of white crystals, m.p. 98–101°. $\nu_{max.}^{KBr}$ 3550, 1736, 1721 (sh), 1555 (split), 1388, 1196, 875, 764 cm.$^{-1}$.

Anal.—Calcd. for $C_{13}H_{20}N_2O_{12}$: C, 39.43; H, 5.09; N, 7.07; CH_3O—, 7.83. Found: C, 39.92; H, 4.48; N, 7.27; CH_3O—, 7.09.

The compound consumed 2.15 equivalents of base (calcd. 2.00 equiv.). Repeated crystallizations did not change the melting point.

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 chromatographed on a thin layer of silica gel. The hydrolysate showed R_f 0.20; a sample of β-methyl-D-glucoside chromatographed on the same plate gave R_f 0.19. The coincidence of these R_f values indicates only that the unknown was a glucose monomethyl ether.

REFERENCES

(1) Finnegan, R. A., Mueller, W. H., and Morris, M. P., *Proc. Chem. Soc.*, **1963**, 182.
(2) Warmke, H. E., Freyre, R. H., and Morris, M. P., *Agron. J.*, **44**, 517(1952).
(3) Nordfeldt, S., *et al.*, *Univ. Hawaii, Agr. Expt. Sta. Tech. Bull. 15*, July 1952.
(4) Emmel, M. W., and Ritchey, G. E., *J. Am. Soc. Agron.*, **33**, 675(1941).
(5) Morris, M. P., Pagan, C., and Warmke, H. W., *Science*, **119**, 322(1954).
(6) Cooke, A. R., *Arch. Biochem. Biophys.*, **55**, 114(1955).
(7) Carter, C. L., and McChesney, W. J., *Nature*, **164**, 575(1949).
(8) Gorter, K., *Bull. Jard. Bot. Buitenzorg*, **2**, 187(1920).
(9) Carrie, M. S., *J. Soc. Chem. Ind. London*, **53**, 288T (1934).
(10) Carter, C. L., *ibid.*, **62**, 238T(1943).
(11) Carter, C. L., *J. Sci. Food Agr.*, **2**, 54(1951).
(12) Gottlieb, O. R., and Magalhaes, M. T., *J. Org. Chem.*, **24**, 2070(1959).
(13) *cf.* Pailer, M., *Fortschr. Chem. Org. Naturstoffe*, **18**, 55(1960).
(14) Rosenberg, M. M., and Zoebisch, O. C., *Agron. J.*, **44**, 315(1952).
(15) Hutton, E. M., Windrum, G. M., and Kratzing, C. C., *J. Nutr.*, **64**, 321(1958); **65**, 429(1958).
(16) Coleman, R. G., Windrum, G. M., and Hutton, E. M., *ibid.*, **70**, 267(1960).
(17) Karrer, W., "Konstitution und Vorkommen der organischen Pflanzenstoffe," Birkhauser, Basel and Stuttgart, 1958, pp. 41, 295, 237.
(18) Gresham, T. L., *et al.*, *J. Am. Chem. Soc.*, **74**, 1323 (1952).
(19) Pailer, M., and Nowotny, K., *Naturwiss.*, **45**, 419 (1958).
(20) Finnegan, R. A., and Mueller, W. H., unpublished data.
(21) Bush, M. T., Goth, A., and Dickison, H. L., *J. Pharmacol.*, **84**, 262(1945).
(22) Bush, M. T., Touster, O., and Brockman, J. E., *J. Biol. Chem.*, **188**, 865(1951).
(23) Nakamura, S., and Shimoda, C., *J. Agr. Chem. Soc. Japan*, **28**, 909(1954); through *Chem. Abstr.*, **50**, 15723 (1956).
(24) Raistrick, H., and Stossl, A., *Biochem. J.*, **68**, 647 (1958).
(25) Birch, A. J., *et al.*, *Chem. Ind. London*, **1960**, 340.
(26) Hylin, J. W., and Matsumoto, H., *Arch. Biochem. Biophys.*, **93**, 542(1960).
(27) Shaw, P. D., and Wang, N., *J. Bacteriol.*, **88**, 1629 (1964).
(28) Gatenbeck, S., and Forsgren, B., *Acta Chem. Scan.*, **18**, 1750(1964).
(29) Dische, Z., "Methods in Carbohydrates," vol. I, Whistler, R. L., and Wolfram, M. L., eds., Academic Press Inc., New York, N. Y., 1962, p. 478.
(30) Bollenback, G. N., "Methyl Glucoside," Academic Press Inc., New York, N. Y., 1958.
(31) Wattiez, M. N., *Bull. Soc. Chim. Biol.*, **7**, 917(1925); **8**, 501(1926); Wattiez, M. N., *J. Pharm. Belg.*, **7**, 81(1925); Plouvier, M. V., *Compt. Rend.*, **256**, 1397(1963).
(32) Nakanishi, K., "Infrared Absorption Spectroscopy—Practical," Holden-Day, Inc., San Francisco, Calif., 1962, p. 34.
(33) Noller, C. R., "Chemistry of Organic Compounds," W. B. Saunders Co., Philadelphia, Pa., 1951, p. 255.
(34) Dannley, R. L., and Kitko, F. V., *Anal. Chem.*, **32**, 1682(1960).
(35) Gillam, A. E., and Stern, E. S., "An Introduction to Electronic Absorption Spectrometry in Organic Chemistry," Edward Arnold Ltd., London, England, 1957, p. 65.
(36) Finnegan, R. A., Stephani, R. A., and Mueller, W. H., to be published.
(37) Kuhn, R., *Angew. Chem.*, **67**, 32(1955).
(38) Neeman, M., *et al.*, *Tetrahedron*, **6**, 35(1959); Neeman, M., and Hashimoto, Y., *J. Am. Chem. Soc.*, **84**, 2972 (1962).
(39) Birkinshaw, J. H., and Dryland, A. M. L., *Biochem. J.*, **93**, 478(1964).