temperature for 20 min. The usual working up yielded 30 mg of the liquid hydrocarbon (XIII): $C_{20}H_{36}$, $[\alpha]_D^{20}$ −27° (c 4.7). IR spectrum (film) (cm^{-1}): 3060, 1645, 890 (> C = CH_2).

Preparation of the Ketone (XV). A mixture of 60 mg of the hydrocarbon (XIII) and 100 mg of OsO_4 in 3 ml of absolute ether was left at room temperature for 48 h. Then it was treated as described for vulgarol. The diol isolated (50 mg) was oxidized with periodic acid (150 mg) in methanol at room temperature for 4 h. The product obtained (40 mg) was chromatographed on silica gel (2 g). A mixture of petroleum ether and 2.5% of benzene eluted 15 mg of the ketone $C_{19}H_{34}O$ (XV) in the form of a mobile liquid. IR spectrum (CCl_4): 1715 cm^{-1} (> C = O). ORD: $[\alpha]_{500}$ −23°, $[\alpha]_{400}$ −38°, $[\alpha]_{345}$ −96°, $[\alpha]_{323}$ −233°, $[\alpha]_{317}$ −169°, $[\alpha]_{313}$ −189°, $[\alpha]_{305}$ −65°, $[\alpha]_{303}$ −60°, $[\alpha]_{299}$ 0°, $[\alpha]_{296}$ + 51°, $[\alpha]_{294}$ +57°, $[\alpha]_{289}$ + 113°, $[\alpha]_{281}$ + 124° (c 0.251, heptane). The ketone (XVI) was obtained from the known hydrocarbon (XIV) in a similar manner. ORD: $[\alpha]_{500}$ −94°, $[\alpha]_{370}$ −169°, $[\alpha]_{245}$ −283°, $[\alpha]_{320}$ −948°, $[\alpha]_{314}$ −712°, $[\alpha]_{310}$ −807°, $[\alpha]_{302}$ −179°, $[\alpha]_{300}$ −141°, $[\alpha]_{297}$ 0°, $[\alpha]_{290}$ + 491°, $[\alpha]_{281}$ + 844°, $[\alpha]_{273}$ + 901° (c 0.212, heptane).

SUMMARY

The structure and stereochemistry of a new minor diterpenoid, vulgarol, isolated from the plant Marrubium vulgare L. has been shown. It has been established that it belongs to the bicyclic diterpenoids with the cis (5β, 10β) linkage of the A/B rings.

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TRITERPENE GLYCOSIDES OF Acanthophyllum gypsophiloides

IV. THE STRUCTURE OF ACANTHOPHYLLOSIDES B AND C

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UDC 547.918:547.914.4

In a preceding paper [1], we reported the structure of the O-glycosidic carbohydrate chains of acanthophyllosides B (I) and C (II). In the present paper we give proof of the structure of the O-acyloside carbohydrate chains of these glycosides. The acyloside carbohydrate chains of the two glycosides are identical and include three molecules of D-xylose, two molecules of O-rhamnose, and one molecule of D-fucose (GLC).

The alkaline saponification of compounds (I) and (II) yielded their progenins, which proved to be identical [1], and the same oligosaccharide. The acid hydrolysis of the oligosaccharide gave D-xylose and L-rhamnose. The absence of D-fucose from the hydrolyzate showed its direct linkage with the carboxy group of the aglycone.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 728-734, November-December, 1975. Original article submitted July 22, 1974.

By the methylation of acanthophylloside B (or C) by Hakomori's method, in the usual way we obtained a mixture of substances with similar R_f values among which the permethylate of acanthophylloside B (III) (or C, (IV)) was present in predominating amount. Of such products, our attention was attracted by compound (V), present in the mixture in very small amounts, but differing from the others in the color which it gave when treated on the chromatogram. After accumulation, it was found that this substance is crystalline, and it was decided to investigate it in detail (Scheme 1).

When the permethylate of acanthophylloside B (III) was subjected to methanolysis followed by hydrolysis of the methyl glycosides formed, the following sugar derivatives were identified: 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-O-arabinose, 3-O-methyl-D-glucuronic acid, 3-O-methyl-D-fucose, 2,3,4-tri-O-methyl-L-rhamnose, 2,3-di-O-methyl-L-rhamnose, 2,4-di-O-methyl-D-xylose, and 2,3,4-tri-O-methyl-D-xylose.

The results of the analysis of the composition of the products obtained in the hydrolysis of the permethylate of (III) and the permethylate of a gypsogenin tetraoside [1] showed that the first three methylated sugars were formed from the O-glycosidic part of the molecule and the last five from the O-acylosidic part. The presence of 3-O-methyl-D-fucose among the methylated monosaccharides meant that the D-fucose is a center of branching, and the presence of completely methylated D-xylose and L-rhamnose showed the terminal position of the corresponding monosaccharides in the carbohydrate chain.

In a hydrolyzate of the permethylate of acanthophylloside C (IV), in addition to the sugars mentioned 2,3,4,6-tetra-O-methyl-D-glucose was also identified.

The IR spectrum of compound (V) lacked the absorption band of an OH group. The number of methoxy groups found from the integral intensity of their protons in the NMR spectrum was 13.8. On complete acid hydrolysis this product decomposed into 3-O-methyl-D-fucose, 2,3,4-tri-O-methyl-L-rhamnose, 2,3-di-O-methyl-L-rhamnose, 2,4-di-O-methyl-D-xylose (2 moles), and 2,3,4-tri-O-methyl-D-xylose. Consequently, substance (V) is the methyl glycoside of the methylated acylosidic oligosaccharide. It was formed by the splitting off of the acyloside chain during the methylation of acanthophyllosides B and C. No such phenomenon has been reported previously in the study of triterpene glycosides.

In the hydrolysis of the methylated oligosaccharide (V) with 0.2% hydrochloric acid, the terminal tri-O-methyl-D-xylose was first split off and then 2,4-di-O-methyl-D-xylose. This shows the direct linkage of the xyloses to one another.

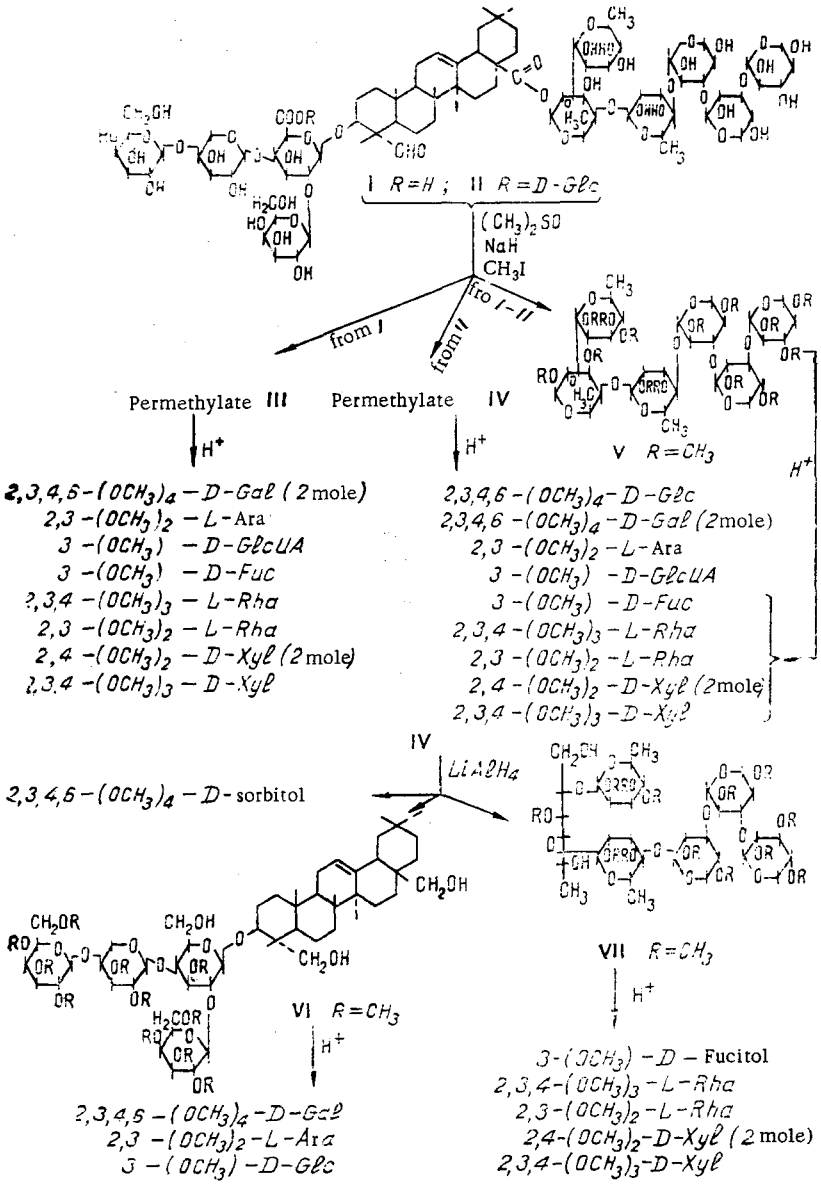
The permethylate (IV) was subjected to reductive cleavage with lithium tetrahydroaluminate. Two substances were isolated: the methylated reduced glycoside (VI) – a tetraoside of 23-hydroxyerythrodiol – and the methylated oligosaccharide (VII). The sugar chain of the glycoside (VI) included 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-L-arabinose and, formed from glucuronic acid, 3-O-methyl-D-glucose.

The methylated oligosaccharide (VII) split off from the carboxy group of the aglycone during reductive cleavage included 3-O-methyl-D-fucitol, 2,3,4-tri-O-methyl-L-rhamnose, 2,3-di-O-methyl-L-rhamnose, 2,4-di-O-methyl-D-xylose, and 2,3,4-tri-O-methyl-D-xylose. The conversion of 3-O-methyl-D-fucose into 3-O-methyl-D-fucitol during reductive cleavage once again confirmed that the fucose is bound directly to the carboxyl of the aglycone. (See Scheme 1 on next page.)

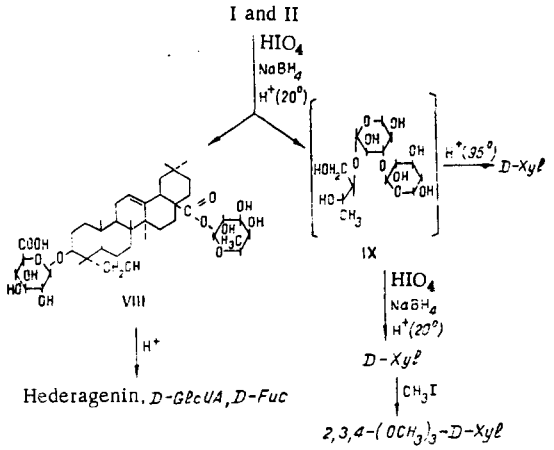
The tetra-O-methyl-D-glucose entering into the composition of the permethylate (IV) was not found in products (VI) and (VII), which shows its direct linkage to the carboxy group of the glucuronic acid. In reductive cleavage, the D-glucose is split off and is reduced to 2,3,4,6-tetra-O-methylsorbitol (see Scheme 1). Then the structure of the O-acylosidic sugar chain of acanthophylloside B and C was refined by two Smith degradations [2]. For this purpose, acanthophyllosides B and C were first oxidized with periodic acid, the hydroxy aldehydes formed were reduced to polyols with sodium tetrahydroborate, and then the products obtained were subjected to mild hydrolysis. In both cases, the degradation led to the isolation of the glycoside (VIII), which precipitated. It follows from the hydrolysis of compound (VIII) that it is a hederagenin glycoside the carbohydrate part of which consists of D-glucuronic acid and D-fucose. The hederagenin was formed from gypsogenin in the reduction of the latter by sodium tetrahydroborate.

The analysis of molecular rotation differences between hederagenin D-glucuronopyranoside and the diglycoside (VIII) showed that the D-fucopyranose is attached to the carboxy group of the hederagenin by a β-glycosidic bond. The formation of compound (VIII) is one of the key points in the establishment of the structure of acanthophyllosides B and C. It not only shows that D-glucuronic acid and D-fucose are attached directly to the aglycone, but that these sugars are centers of branching. (See Scheme 2).

Scheme 1


I R=H ; II R=D-Glc
(CH3)2SO
NaH
CH3I
from I
from II
from I-II
Permethylate III
Permethylate IV
H+
V R=CH3
2,3,4,6-(OCH3)4-D-Gal (2 mole)
2,3-(OCH3)2-L-Ara
3-(OCH3)-D-GlcUA
3-(OCH3)-D-Fuc
2,3,4-(OCH3)3-L-Rha
2,3-(OCH3)2-L-Rha
2,4-(OCH3)2-D-Xyl (2 mole)
2,3,4-(OCH3)3-D-Xyl
2,3,4,6-(OCH3)4-D-Glc
2,3,4,6-(OCH3)4-D-Gal (2 mole)
2,3-(OCH3)2-L-Ara
3-(OCH3)-D-GlcUA
3-(OCH3)-D-Fuc
2,3,4-(OCH3)3-L-Rha
2,3-(OCH3)2-L-Rha
2,4-(OCH3)2-D-Xyl (2 mole)
2,3,4-(OCH3)3-D-Xyl
IV
LiAlH4
2,3,4,6-(OCH3)4-D-sorbitol
VI R=CH3
2,3,4,5-(OCH3)4-D-Gal
2,3-(OCH3)2-L-Ara
3-(OCH3)-D-Glc
VII R=CH3
3-(OCH3)-D-Fucitol
2,3,4-(OCH3)3-L-Rha
2,3-(OCH3)2-L-Rha
2,4-(OCH3)2-D-Xyl (2 mole)
2,3,4-(OCH3)3-D-Xyl

Scheme 2



After the first Smith degradation and the isolation of the glycoside (VIII), the reaction mixture should presumably contain the polyol (IX) (Scheme 2). In this only the two intermediate D-xylose molecules, connected in acanthophyllosides B and C by a 1→3 bond, should have remained unaffected. On periodate oxidation and subsequent reduction, the rhamnose attached to the xylose is converted into a diol resistant to acid cleavage at room temperature. On the other hand, the alcohol fragment formed from the terminal xylose is labile under the action of acid and is readily cleaved under the conditions of the reaction [2]. One part of the reaction mixture was hydrolyzed with heating, which led to the complete decomposition of the hypothetical compound (IX) into individual fragments, and D-xylose was detected in the reaction mixture. The second Smith degradation of the other part of the reaction mixture oxidized the second terminal D-xylose molecule. The oxidation product was methylated by Hakomori's method, and 2,3,4-tri-O-methyl-D-xylose was identified by TLC in a hydrolyzate of the permethylate (see Scheme 2).

It follows from the twofold Smith degradation that to the D-fucose at the C_2 and C_4 hydroxyls are attached to two molecules of L-rhamnose, one of which has at its fourth hydroxyl a chain of three D-xylose molecules linked to one another by 1→3 bonds.

Thus, acanthophyllosides B and C must correspond to structures (I) and (II), respectively. The proposed structural formulas correspond to the experimental material that we have adduced. The positions of the bonds of the terminal L-rhamnose and of the tetrasaccharide residue (L-rhamnose + 3 D-xyloses) with the D-fucose molecule remain uncertain: they should possibly be interchanged. The terminal L-rhamnose may be attached not to the hydroxy group at C_2 of the D-fucose as is shown in the scheme, but to the hydroxyl at C_4. In the latter case, the tetrasaccharide residue must substitute position 2 of the D-fucose.

Acanthophyllosides B and C are the most complex of the triterpene glycosides of plants of the family Labiatae known to us (see Schemes 1 and 2).

EXPERIMENTAL

For chromatography we used type M ("slow") paper, KSK silica gel, and the following solvent systems: 1) chloroform–methanol–water (65 :35 :8); 2) chloroform–methanol–water (65 :35 :10); 3) butan-1-ol–methanol–water (5 :3 :1); 4) butan-1-ol–acetic acid–water (4 :1 :5); 5) butanol–pyridine–water (6 :4 :3); 6) benzene–acetone (2 :1); 7) chloroform–methanol (6 :1); 8) butan-1-ol–ethanol–water (5 :1 :4); 9) chloroform–methanol (25 :1); 10) chloroform–methanol (10 :1); 11) toluene–methanol (9 :1); 12) chloroform–methanol (8 :1); 13) water-saturated methyl ethyl ketone.

The free monosaccharides were chromatographed on plates bearing silica gel impregnated with a 0.3-ml solution of NaH_2PO_4. The glycosides were revealed with a solution of phosphotungstic acid, and the sugars with o-toluidine salicylate.

Gas–liquid chromatography of the silylated methyl glycosides was performed on a UKh-1 chromatograph using a copper column (1 m × 4 mm) containing 5% of G-30 m silicone phase on Diaforit (0.2–0.315 mm) at a column temperature of 176°C with hydrogen as the carrier gas at the rate of flow of 55 ml/min.

The positions of the free hydroxy groups in the partially methylated sugars were determined from a comparison of the results of their periodate oxidation and of color reactions with triphenyltetrazolium chloride and diphenylamine.

Methylation of Acanthophyllosides B (I) and C (II). Glycoside B (1 g) in 30 ml of dimethyl sulfoxide was methylated by the addition of 1 g of sodium hydride and 5 ml of methyl iodide. The course of the reaction was monitored by TLC in systems 6 and 11, and in addition to the main product – the permethylate of acanthophylloside B (III) – among the violet-colored methylation by products was found a very small amount of substance (V), which appeared in the form of a yellow spot.

The mixture of methylated products (0.8 g) obtained after the usual treatment, was separated on a column of silica gel in system 6. The chromatographically homogeneous amorphous permethylate of glycoside B (III) (0.6 g) and the crystalline products (V) with mp 208-210°C (from methanol), $[\alpha]_D^{20}-24 \pm 1°$ (c 1.89; absolute methanol) were isolated. The IR spectrum of substance (V) lacked the absorption band of an OH group, and the NMR spectrum showed the presence of 13.8 OCH_3 groups (from the number of CH_3-group protons). Calculated for the methyl glycoside of the methylated hexasaccharide, 14 OCH_3 groups.

Under similar conditions, acanthophylloside C gave the permethylate (IV) and compound (V).

Hydrolysis of the Permethylates B (III) and C (IV). The permethylate (III) (0.2 g) was boiled in 30 ml of 7% methanolic $HClO_4$ solution for 6 h. Then the solution was diluted with an equal volume of water, the methanol was distilled off, and the residual solution was heated for another 3 h. The precipitate that deposited was filtered off, and the hydrolyzate was neutralized with Dowex 1×2 ion-exchange resin (OH^- form) and concentrated. The following methylated sugars were found in the hydrolyzate by PC (system 13) and TLC (system 12) in the presence of markers: 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-L-arabinose, 3-O-methyl-D-glucuronic acid, 3-O-methyl-D-fucose, 2,3,4-tri-O-methyl-L-rhamnose, 2,3-di-O-methyl-L-rhamnose, 2,4-di-O-methyl-D-xylose, and 2,3,4-tri-O-methyl-D-xylose.

The permethylate of acanthophylloside C (IV) was hydrolyzed under the same conditions. In the hydrolyzate, all the methylated monosaccharides mentioned above for acanthophylloside B were identified and, in addition, 2,3,4,6-tetra-O-methyl-D-glucose.

Hydrolysis of Compound (V). The hydrolysis of 10 mg of the substance was performed as described for (III). It was shown by TLC (systems 6 and 12) and PC (system 13) that the hydrolyzate contained 3-O-methyl-D-fucose, 2,3,4-tri-O-methyl-L-rhamnose, 2,3-di-O-methyl-L-rhamnose, 2,4-di-O-methyl-D-xylose, and 2,3,4-tri-O-methyl-D-xylose.

Partial Hydrolysis of Compound (V). Compound (V) (10 mg) was heated in 3 ml of 0.2% aqueous methanolic HCl at 90°C for 5 h. The process was monitored by TLC (system 6) and PC (system 13) every hour. During the first hour the appearance of 2,3,4-tri-O-methyl-D-xylose was observed. In the following hour 2,4-di-O-methyl-D-xylose was split off. On further heating for 3 h, no other methylated sugars were formed.

Reductive Cleavage of the Permethylates (III) and (IV) of Acanthophyllosides B and C. A solution of 0.2 g of the permethylate (IV) in 15 ml of absolute tetrahydrofuran was treated with 1 g of lithium tetrahydroaluminate and boiled with stirring for 8 h. The excess of $LiAlH_4$ was decomposed with ethyl acetate and then with water. After the addition of sufficient 5% sulfuric acid to dissolve the precipitate completely, the reaction mixture was extracted with ether. The combined extracts were washed with water and evaporated to dryness. The dried residue was separated on a column of silica gel in system 6. This gave ~30 mg of glycoside (VI). After the hydrolysis of compound (VI) with 5% sulfuric acid in methanol., an aglycone was isolated which was identified by TLC in systems 9 and 10 as 23-hydroxyerythrodiol. In the hydrolyzate after neutralization with $BaCO_3$, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-L-arabinose, and 3-O-methyl-D-glucose were detected by PC (system 13) and TLC (systems 6 and 7).

The acidic aqueous solution after ethereal extraction was neutralized with Dowex 1 × 2 anion-exchange resin (100-200 mesh) (OH^- form) and extracted with chloroform–ethanol (1 : 1). The combined extracts were washed with water, the solvent was distilled off, and the residue was purified on a column of silica gel in system 6. The chromatographically individual reduced methylated oligosaccharide (VII) was hydrolyzed under the conditions given above, and completely-methylated rhamnose and xylose and 2,4-di-O-methyl-D-xylose, 2, 3-di-O-methyl-L-rhamnose, 3-O-methyl-D-fucitol, and 2,3,4,6-tetra-O-methyl-D-sorbitol were identified by PC (system 13) and TLC (system 6) with markers, the spots being revealed on the chromatograms with Bonner's reagent.

The reductive cleavage of the permethylate of (III) gave the same results, with the exception that 2,3,4, 6-tetra-O-methyl-D-sorbitol was absent from all the reaction products.

Smith Degradation of Glycosides B and C. Acanthophylloside C (1 g) was dissolved in 100 ml of 4% aqueous periodic acid and was oxidized at 5°C for 24 h. The reaction was monitored by the TLC method (sys-

tem 1) until the initial compound had disappeared. Then the solution was neutralized with $BaCO_3$, the precipitate that deposited was filtered off, and the filtrate was treated with 1.0 g of $NaBH_4$ and was left at room temperature for 15 h. The reaction mixture was neutralized with acetic acid and concentrated to 24 ml, and then 1.3 ml of concentrated HCl was added to it and it was left at 20°C for 6 h. The precipitate that had deposited (500 mg) was filtered off. The filtered hydrolyzate was neutralized with $NaHCO_3$ and extracted with butanol. The butanolic extract was washed with water and evaporated to dryness, and the residue was combined with the precipitate that had deposited.

The mixture of substances obtained (500 mg) was chromatographed on a column of silica gel in system 2. This yielded 150 mg of hederagenin 28-O-fucopyranoside-3-O-β-D-glucopyranoside (VIII), $C_{42}H_{66}O_{14}$, mp 260-265°C, $[\alpha]_D^{20}$ + 9.9 ± 2° (c 0.488; methanol). The aqueous fraction after extraction with butanol was evaporated. This gave 100 mg of dry residue (25 mg of which was hydrolyzed with 5% sulfuric acid (95°C, 5 h). The hydrolyzate was neutralized with barium carbonate, and D-xylose was identified by TLC (system 3).

The remaining product (75 mg) was subjected to a second Smith degradation under the conditions described above.

The product of the second degradation was methylated by Hakomori's method as described for acanthophylloside B. The permethylate after methanolysis with 7% HCl and subsequent hydrolysis gave 2,3,4-tri-O-methyl-D-xylose, identified by TLC in system 6.

The Smith degradation of acanthophylloside B gave the same products.

Hydrolysis of Compound (VIII). A mixture of 0.02 g of compound (VIII) and 5% H_2SO_4 in aqueous methanol (3 : 1) was heated at 95°C for 5 h. The precipitate that deposited on hydrolysis was identified by the TLC method (system 9 and 10) as hederagenin.

D-Glucuronic acid and D-fucose were found by TLC (system 3) in the hydrolyzate neutralized with $BaCO_3$.

Methylation of Compound (VIII). Compound (VIII) was methylated as described for acanthophylloside B. The sugars split off in the hydrolysis of the permethylate of the glycoside (VIII) were shown by thin-layer chromatography in systems 6 and 12 to be 2,3,4-tri-O-methyl-D-fucose and 2,3,4-tri-O-methyl-D-glucuronic acid.

SUMMARY

Alternative structures for the acyloside chain of acanthophylloside B and C have been established.

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