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STRUCTURE OF DICHROSIDE D, A FATTY ACID GLYCOSIDE FROM *Ipomoea dichroa**

DARWIN A. HARRISON, KUNNATH P. MADHUSUDAN, AND DINESH K. KULSHRESHTHA**
Medicinal Chemistry Division, Central Drug Research Institute, Lucknow (India)
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ABSTRACT

Four fatty acid glycosides, designated dichrosides A, B, C, and D, have been isolated from *Ipomoea dichroa* (family, Convolvulaceae). On chemical investigation, dichroside D was found to be a pentaglycoside of 11-hydroxyhexadecanoic acid, and the sugar chain is highly esterified with other fatty acids. The structure of the pentaglycoside has been established as 11-{*O*-β-D-glucopyranosyl-(1→4)-*O*-[β-D-fucopyranosyl-(1→3)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl)oxy}hexadecanoic acid.

INTRODUCTION

A survey of the literature[1–11] showed that the genus *Ipomoea* is rich in fatty acid glycosides. Mono- and di-hydroxy C_{14}, C_{15}, and C_{16} fatty acids are present as aglycons, whereas glucose, fucose, rhamnose, and quinovose constitute the sugar moiety. Occasionally, the sugar chain is esterified with lower acids[3], and the glycosidic acids are esterified with each other to form a mixture of closely related polyesters.

The ethanolic extractive of *Ipomoea dichroa* Chois (Convolvulaceae) showed tumor inhibitory activity in the KB cell-culture system (ED_{50} 20 μg/mL). Because there was no previous report on chemical studies of this plant, it was chosen for chemical investigation. This led to the isolation of four fatty acid glycosides, which were designated dichrosides A, B, C, and D. The present communication deals with elucidation of the structure of dichroside D.

RESULTS AND DISCUSSION

The ethanolic extractive of the dried plant (aerial parts) was resolved into hexane-, chloroform-, 1-butanol-, and water-soluble fractions. The hexane fraction was found to be rich in fatty acid glycosides. This fraction was therefore repeatedly

*C.D.R.I. Communication No. 3541.
**To whom correspondence should be addressed.

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chromatographed and dichrosides A, B, C, and D were isolated. Dichroside D was the major component, and showed significant anticancer activity in the KB cell-culture system (ED_{50} 8.4 μg/mL). It was therefore chosen for structure elucidation.

Dichroside D was obtained as an amorphous solid which showed a single spot in t.l.c. and u.v. absorption at 280 nm. As dichroside D was found to be highly non-polar, being ether-soluble, it was considered to be highly esterified. It was, therefore, subjected to alkaline hydrolysis, whereupon an intractable mixture was obtained. Dichroside D was also found to be unstable on keeping for a few days at room temperature. On catalytic hydrogenation, however, dichroside D lost its instability, and the product did not exhibit absorption at λ_{max} 280 nm. Further work was therefore conducted with the hydrogenation product, henceforth referred to as dichroside D_1.

Dichroside D_1 was hydrolyzed with ethanolic hydrochloric acid, and the neutralized hydrolyzate was resolved into chloroform-soluble and aqueous portions. The chloroform-soluble portion contained the aglycon. On chromatography of the aglycon mixture, only the major product could be isolated. From i.r., n.m.r., e.i.–m.s., and CH_4-c.i.–m.s. studies, it was characterized as the ethyl ester of 11-hydroxyhexadecanoic acid. Its identity was further confirmed by mass-spectral analysis of its trimethylsilyl ether. G.l.c.–m.s. analysis of the aglycon mixture, however, furnished mass spectra for the ethyl esters of a C_{12} acid [*m/z* 228 (M^+), R_T 2.1 min] and an unsaturated C_{16} acid [*m/z* 282 (M^+), R_T 8.2 min]. The latter was obviously formed by dehydration of ethyl 11-hydroxyhexadecanoate during passage through the g.l.c. column.

Dichroside D_1 was subjected to methanolysis[12] with 0.05% MeONa for 1.5 h, and the resulting product was steam-distilled. The distillate was found, by mass-spectral analysis of the distillate, to contain the methyl ester of α-methylbutanoic

$$\overset{+OH}{\underset{\|}{\dot{C}}}H-COCH_3$$

acid, *m/z* 101 (M − 15), 88 (CH_3–$\dot{C}H$–$COCH_3$), 87, and 85. The nonvolatile fraction was partitioned between water and chloroform. On g.l.c.–m.s. analysis, the chloroform-soluble fraction showed the presence, as their methyl esters, of C_{12}, C_{14}, C_{16}, and C_{18} acids, and a C_{18} acid having a double bond. This double bond must have arisen by dehydration of the methyl ester of a C_{18} hydroxy acid during its passage through the g.l.c. column, as no unsaturation would be expected in the acid derived from dichroside D_1, the hydrogenation product of dichroside D.

The water-soluble fraction of the methanolysis product showed, in t.l.c., two spots designated dichrosides D_2 and D_3 in the decreasing order of their R_F values. Dichrosides D_2 and D_3 were separated, and purified, by column chromatography. However, further treatment of dichroside D_2 with 0.05% MeONa for 3 h led to the formation of D_3 along with the liberation of a few more acids, which, by g.l.c. of their methyl esters were identified as C_{12}, C_{14}, C_{16}, and C_{18} acids.

On hydrolysis, first with 90% HCO_2H and then with 0.1M aq. H_2SO_4 at 100°,

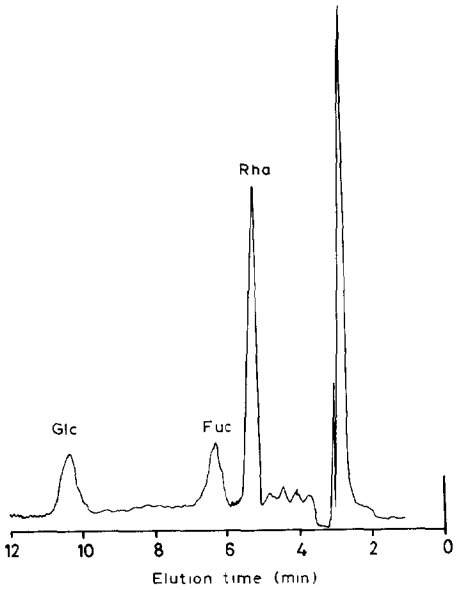


Fig. 1. Chromatogram from l.c. analysis of the sugars obtained from dichroside D_3 on "Carbohydrate Analysis" column (Waters Associates). [Eluant, 17:3 acetonitrile–water; flow rate, 1.0 mL per min; refractive-index detection.]

dichroside D_3 furnished a mixture of glucose, fucose, and rhamnose, identified by p.c. The ratios of the sugars were established as Glc:Fuc:Rha = 1:1:3 by l.c. (see Fig. 1). The aglycon precursor was, by co-injection in g.l.c. with the aglycon of dichroside D_1 and combined g.l.c. and CH_4-c.i.-m.s., identified as 11-hydroxyhexadecanoic acid.

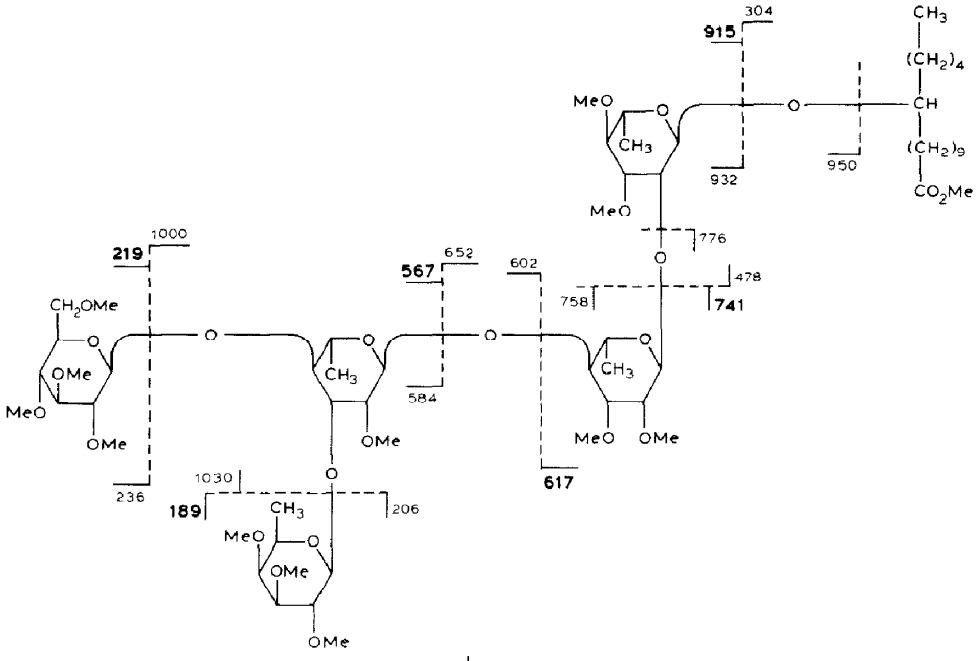
As, on alkaline methanolysis, dichroside D_1 liberated fatty acids, finally giving rise to dichroside D_3, it was evident that these acids must be attached to the non-anomeric hydroxyl groups of the sugar moiety through ester linkages, the anomeric hydroxyl group of the reducing-end sugar being substituted by the aglycon. This was verified by treating the aqueous portion of the acid hydrolyzate of dichroside D_1, containing the esterified sugars, with 0.05% MeONa. After neutralization of the base, the hydrolyzate was partitioned between $CHCl_3$ and water. The $CHCl_3$-soluble mixture was found, by g.l.c. analysis, to contain the methyl esters of C_{12}, C_{14}, C_{16}, and C_{18} acids. From the aqueous portion, free sugars were isolated by chromatography on silica gel, and their specific rotations were determined at anomeric equilibrium; they were characterized as D-glucose, $[\alpha]_D$ +46°; D-fucose, $[\alpha]_D$ +71°; and L-rhamnose, $[\alpha]_D$ +8.7°.

In the original dichroside D, some of these acids must carry the unsaturation responsible for the u.v. absorption. That the double bonds were not present in the 11-hydroxyhexadecanoic acid was inferred from the fact that the same acid was obtained on acid hydrolysis both of dichroside D and its hydrogenation product, dichroside D_1.

Structure of the sugar moiety. — In order to bring about selective cleavage of the sugar chain by mild hydrolysis with acid, dichroside D_1 was treated with 1% hydrochloric acid in dry 1,4-dioxane, and liberation of sugars was monitored by p.c. in 1-butanol saturated with water. The first sugar to appear in p.c., after 30 min of hydrolysis, was glucose. Later, a mixture of sugars started to appear. This indicated that glucose was a terminal sugar in the carbohydrate chain. Other methods of selective hydrolysis did not yield satisfactory results.

Further, dichroside D_1, along with D_2 and D_3, was subjected to permethylation by the Hakomori method[13], and the permethylated glycosides were hydrolyzed with acid. The resulting partially methylated sugars were converted into their hexitol acetates, and these analyzed by g.l.c.–m.s. and g.l.c. according to the method of Lindberg *et al.*[14]. Thus, the hexitol acetates derived from dichrosides D_1, D_2, and D_3 were identified as 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol, and 1,3,4,5-tetra-*O*-acetyl-2-*O*-methylrhamnitol. Because highly alkaline conditions had been employed for the permethylation, ester groups were removed, and replaced by methyl groups; hence, all three dichrosides (D_1, D_2, and D_3) yielded the same hexitol acetates.

Formation of the foregoing methylated hexitol acetates clearly indicated that



1 $[M + NH_4]^+$, *m/z* 1218

Numbers in boldface type refer to ions in e i – m s , and those in normal type refer to NH_4^+ adduct ions in c i – m s

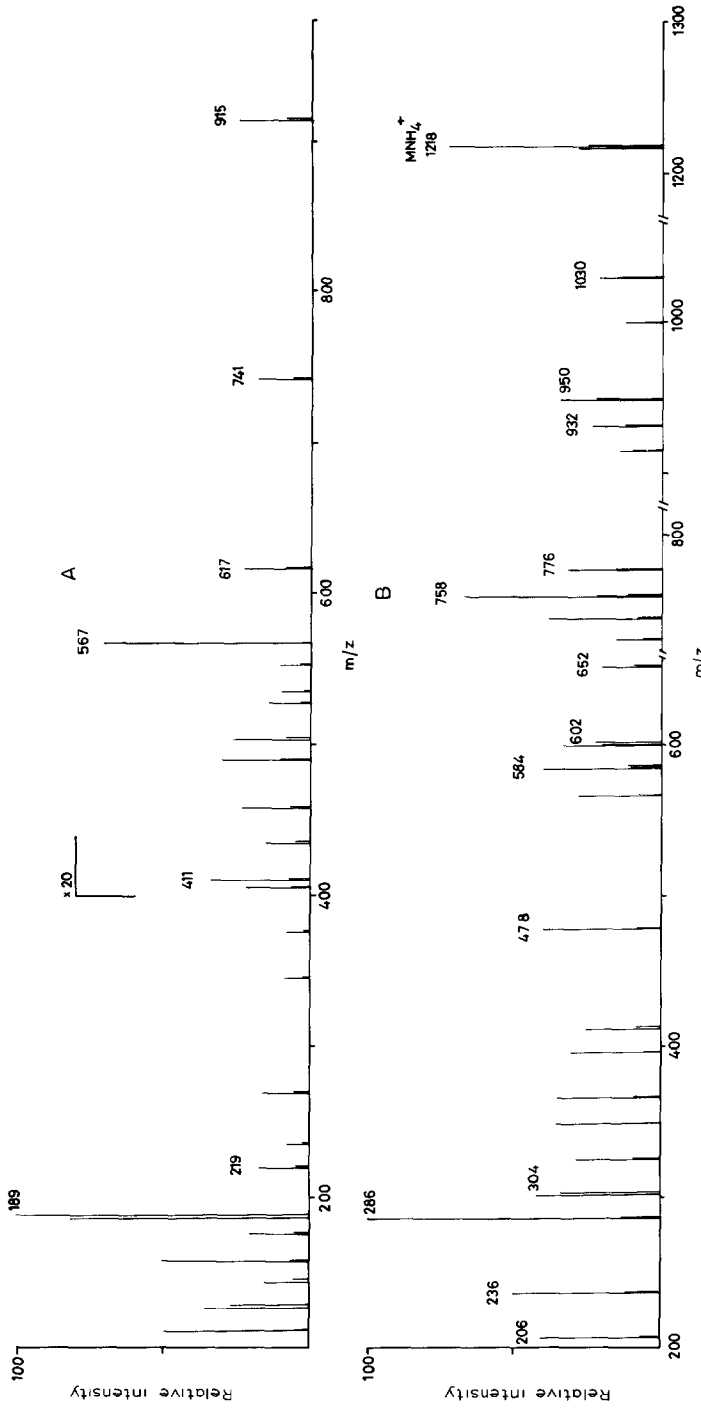
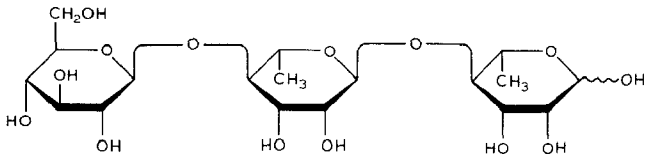


Fig. 2. (A) E.i. mass spectrum of permethylated dichroside D_3 at 20 eV. (B) Ammonia c.i. mass spectrum of permethylated dichroside D_3.

glucose and fucose were the two terminal sugars, joined to the end unit of a sugar chain consisting of three rhamnosyl residues. Further support for the sequence of sugars was forthcoming from the fragmentation pattern in the e.i.–m.s. and NH_3-c.i.–m.s. of permethylated dichroside D_3, as shown in **1** (see Fig. 2).

Because attempts to bring about selective cleavage of the sugar chain by the usual methods (such as acetolysis, and treatment with 1% acetic acid and with formic acid under various conditions) did not yield satisfactory results, further studies were conducted with dichroside D_2. Treatment thereof with 80% aq. formic acid for 2 h at 80° led to the formation of a product of R_F value lower than that of dichroside D_2, along with fucose, and rhamnose (t.l.c.). This product was subjected to methanolysis with MeONa, to give a trisaccharide. The trisaccharide was now hydrolyzed with acid, and paper chromatography of the hydrolyzate showed that it contained only glucose and rhamnose, and that fucose was absent. Glucose and rhamnose were present in the ratio of 1:2, as established by l.c. The trisaccharide was permethylated by the Hakomori method, and this was followed by acid hydrolysis. The partially methylated sugars so formed were identified as 2,3,4,6-tetra-*O*-methylglucose and 2,3-di-*O*-methylrhamnose by g.l.c.–m.s. analysis of their hexitol acetates.

It is clear that both of the rhamnose units present in the trisaccharide yielded only 2,3-di-*O*-methylrhamnose. One of the 2,3-di-*O*-methylrhamnose units must be the same as that derived from dichroside D_2. The other 2,3-di-*O*-methylrhamnose unit must have arisen after liberation of fucose from a rhamnose unit which was responsible for giving rise to 2-*O*-methylrhamnitol. Thus, the trisaccharide could be represented as **2**.



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Formation of **2** revealed that the fucose unit removed from dichroside D_2 during treatment with HCO_2H occupied O-3 of the rhamnosyl residue bearing the glucosyl group at O-4. Furthermore, the rhamnose unit responsible for giving rise to 3,4-di-*O*-methylrhamnose from dichrosides D_1, D_2, and D_3 must be the reducing-terminal unit, with the anomeric carbon in glycosidic union with the 11-oxygen atom of the aglycon.

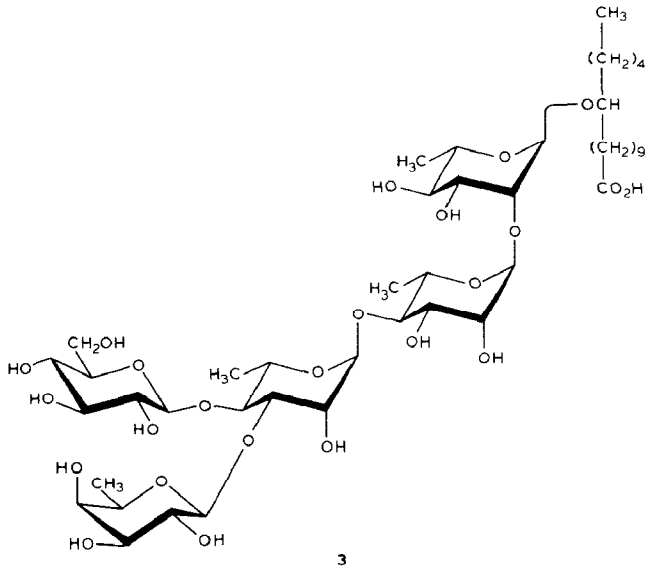
The 400-MHz, ^{1}H-n.m.r.-spectral studies were conducted with dichroside D_2 instead of D_3, as the spectrum of the former was better resolved. Extensive decoupling experiments led to the signal assignments and their coupling patterns recorded in Table I. Although all of the proton signals of the rhamnose units could be recognized, only four signals, *viz.*, those caused by H-1, H-2, H-3, and H-6,

TABLE I

CHEMICAL SHIFTS IN THE 400-MHz, ^{1}H-N M R SPECTRUM OF DICHROSIDE D_2 IN CD_3OD

Atom	*Rhamnose unit*			*Rhamnose unit*			*Rhamnose unit*			*Glucose unit*			*Fucose unit*		
	δ Value	J	*(Hz)*	*δ Value*	J	*(Hz)*	*δ Value*	J	*(Hz)*	*δ Value*	J	*(Hz)*	*δ Value*	J	*(Hz)*
H-1	5.24	d,	2.0	5.22	d,	2.0	4.94	d,	2.0	4.42	d,	7.5	4.31	d,	7.5
H-2	4.02	dd,	2.0, 3.5	3.84	dd,	2.0, 3.5	5.54	dd,	2.0, 3.5	3.15	dd,	7.5, 9.0	3.64	dd,	7.5, 9.0
H-3	3.55	dd,	3.5, 9.0	3.79	dd,	3.5, 9.0	4.06	dd,	3.5, 9.0	—	—	—	3.83	dd,	2.0, 9.0
H-4	3.38	t,	9.0	3.39	t,	9.0	3.63	t,	9.0						
H-5	3.67	m,	7.0, 9.0	4.30	m,	7.0, 9.0	3.86	m,	7.0, 9.0						
H-6	1.11	d,	7.0	1.08	d,	7.0	1.12	d,	7.0				1.11	d,	7.0

could be assigned in the case of fucose. The signals of the glucose units were much too masked, and only the H-1 and H-2 signals could be identified. Nevertheless, the coupling patterns of these sugars clearly indicated that all of the sugars existed in the pyranose form. The L-rhamnose units possessed the $^1C_4$(L) conformation, and were linked through α-linkages, as indicated by $J_{1,2}$ values of 2 Hz, which were consistent with those reported for α-L-rhamnopyranose[15]. D-Glucopyranose and D-fucopyranose units were in the $^4C_1$(D) conformation, and were linked to the rhamnopyranose unit through β-linkages ($J_{1,2}$ 7.5 Hz). Because dichroside D_3 was the de-esterification product of dichroside D_2, the former would thus be represented by structure **3**.



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EXPERIMENTAL

General. — Optical rotations were measured in a 1-dm tube with a Jasco-Dip 180 polarimeter. Gas–liquid chromatography was conducted in a Varian Autoprep 1868 A gas chromatograph, and l.c. in a Waters Associates, model ALC/GPC 244, instrument using a "Carbohydrate Analysis" column. T.l.c. was performed on plates of silica gel G, using the following solvents: (*1*) 8:1:1 ethyl acetate–methanol–water and (*2*) 8:8:1 ethyl acetate–acetone–water. Paper chromatography was conducted on Whatman No. 1 filter paper, using 1-butanol saturated with water. U.v. spectra were recorded with a Perkin–Elmer 202 spectrophotometer, and i.r. spectra with a Perkin–Elmer Infracord 177 instrument. ^{1}H-N.m.r. spectra were recorded with a 90-MHz Perkin–Elmer R-32 spectrometer and a Bruker 400-MHz spectrometer, for solutions in $CDCl_3$, with Me_4Si as the internal standard. Mass spectra were recorded at 20 eV with a JEOL JMS-D300 mass spectrometer

and g.l.c.–m.s. was performed in the same instrument coupled with a gas–liquid chromatograph.

Extraction and isolation of dichrosides A, B, C, and D. — The air-dried, aerial portion (9 kg) of *Ipomoea dichroa* (collected from Lucknow, India, in October, 1978; voucher specimen deposited in the CDRI herbarium) was powdered, and extracted with ethanol (4 × 10 L). The extracts were combined and concentrated under diminished pressure below 50°. The concentrate was then diluted with water, and successively extracted with hexane (4 × 1 L), chloroform (4 × 1 L), and 1-butanol (4 × 1 L), to afford hexane (192 g), chloroform (42 g), 1-butanol (78 g), and water-soluble (90 g) fractions, respectively.

The hexane-soluble fraction showed four spots, of dichroside A, B, C, and D (R_F values 0.63, 0.48, 0.32, and 0.18, respectively) in t.l.c. in solvent *1*. This fraction (190 g) was therefore subjected to gross fractionation in a column of silica gel (2 kg) mixed with hexane. Elution was successively effected with hexane, benzene, ethyl acetate, and ethanol, and fractions (1 L each) were collected. Rechromatography of the material (100 g) from the ethyl acetate eluate on silica gel (1 kg), with ethyl acetate + 10% ethanol as the eluant, afforded a mixture (30 g) of dichrosides A, B, C, and D. This fraction was further chromatographed on silica gel (900 g), leading to the isolation of pure dichrosides B, C, and D. The results of this chromatography are given in Table II.

Dichroside D. — Dichroside D was obtained as a colorless, amorphous solid; $[\alpha]_D$ −26° (*c* 1.0, ethanol); λ_{max} 240, and 280 nm; ν_{max}^{KBr} 3400, 2910, 2840, 1715, 1380, and 1070 cm^{-1}.

Catalytic hydrogenation of dichroside D. — An ethanolic solution (20 mL) of dichroside D (1 g) was stirred with PtO_2 (80 mg) under an atmosphere of H_2 for 5 h. The catalyst was removed by filtration, and the filtrate evaporated to dryness. The residue (dichroside D_1) had $[\alpha]_D$ −20° (*c* 1, ethanol), did not show u.v. absorption at 280 nm, and remained unchanged on being kept for several days.

Methanolysis of dichroside D_1. — To a solution of dichroside D_1 (500 mg) in

TABLE II

CHROMATOGRAPHY OF THE MIXTURE (30 g) OF DICHROSIDES A, B, C, AND D ON SILICA GEL

Fraction (500 mL)	*Eluant*	*Weight (g)*	*Remarks*
1	Ethyl acetate	1.50	oily mixture
2	Ethyl acetate	0.25	dichroside A
3	Ethyl acetate	0.45	dichroside B
4	Ethyl acetate	4.60	dichroside B + C
5	Ethyl acetate	2.00	dichroside C
6	Ethyl acetate	3.50	dichroside C + D
7	Ethyl acetate	5.60	dichroside D + streaking
8	Ethyl acetate	1.80	dichroside D
9	Ethyl acetate	7.00	dichroside D + streaking
10	Ethanol	2.00	streaking

absolute methanol (18 mL) was added 0.5% MeONa (2 mL), the mixture kept for 100 min at room temperature, and ice was added. The base was then neutralized with Amberlite IR-120 (H^+), and the product was steam-distilled. For mass-spectral analysis, a sample of the distillate was placed in a capillary tube, which was inserted into the direct-inlet system of the instrument, and the mass spectrum recorded. The analysis indicated that the steam distillate contained the methyl ester of α-methylbutanoic acid; *m/z* 101 (M − 15), 88 (CH_3–$\overset{+OH}{\overset{\|}{C}}$–$OCH_3$), 87, and 85.

The nonvolatile portion was resolved into chloroform- and water-soluble fractions. The chloroform fraction was subjected to g.l.c.–m.s. Results are recorded in Table III.

The solution of the water-soluble portion was evaporated to dryness. The residue (310 mg) showed two major spots in t.l.c. (solvent *2*) corresponding to dichrosides D_2 and D_3. It was chromatographed on silica gel (30 g), gradient elution being achieved by using ethyl acetate saturated with water containing increasing proportions of acetone. Twenty-eight fractions (20 mL each) were collected. Fractions 6–9, eluted with ethyl acetate saturated with water + 10% of acetone, were evaporated to give colorless, amorphous, solid dichroside D_2 (45 mg). On evaporation of fractions 13–25, eluted with acetone, dichroside D_3 was obtained as a colorless residue (70 mg); $[\alpha]_D$ −62° (*c* 1.0, ethanol).

Acid hydrolysis of dichroside D_1. — Dichroside D_1 (250 mg) was refluxed with 2M HCl in 4:1 ethanol–water (3 mL) for 4 h. It was then cooled, diluted with water (3 mL), and the ethanol evaporated. The aqueous, acidic hydrolyzate was heated on a boiling-water bath for 30 min, cooled, and extracted with dichloromethane (5 × 1 mL). The extracts were combined, successively washed with aqueous $NaHCO_3$ solution and water, and evaporated, to give crude aglycon (98 mg). Evaporation of the aqueous phase gave a residue (145 mg).

The aglycon. — G.l.c.–m.s. in a column of 3% of OV-1 at 225°, of the aglycon mixture furnished the values for ethyl esters of a C_{12} acid [*m/z* 228 (M^+), R_T 2.1 min] and an unsaturated C_{16} acid [*m/z* 282 (M^+), R_T 8.2 min]. The crude aglycon

TABLE III

G L C –M S ANALYSIS OF THE METHYL ESTERS LIBERATED BY TREATMENT OF DICHROSIDE D_1 WITH 0.05% MeONa. COLUMN 3% OF OV-1 AT 225°

Acid or methyl ester	R_T *(min)*	M^+ (m/z)
Methyl ester of C_{12} acid	2.3	214
Methyl ester of C_{14} acid	4.5	242
Methyl ester of C_{16} acid	8.2	270
C_{18} Acid	9.5	284
Methyl ester of C_{18} acid + 1-double-bond acid	10.5	296
Methyl ester of C_{18} acid	10.9	298

(95 mg) was subjected to column chromatography on silica gel (10 g), using benzene as the solvent. Polarity was increased by gradual addition of methanol. Twenty-five fractions (50 mL each) were collected. Fractions 16 and 18, respectively eluted with benzene + 2% of methanol and benzene + 3% of methanol consisted of the ethyl ester of 11-hydroxyhexadecanoic acid (15 mg) and free 11-hydroxyhexadecanoic acid (5 mg), respectively.

Ethyl 11-hydroxyhexadecanoate. — This had $\nu_{max}^{CHCl_3}$ 3300, 2860, 1720, 1450, and 1175 cm^{-1}; ^{1}H-n.m.r. (CCl_4): δ 1.25 (bs, 29 H, $CH_2(CH_2)_{12}$–CH_3), 1.19 (t, J 7 Hz, OCH_2CH_3), 2.16 (t, 2 H, J 7 Hz, $COCH_2$), 3.41 (m, 1 H, $CHOH$), 3.96 (q, 2 H, J 7 Hz, OCH_2CH_3); e.i.–m.s.: m/z 300 (M^+), 255 ($M - OC_2H_5$), 199 $[CH_2(CH_2)_9\overset{\overset{OH}{\|}}{C}OOC_2H_5]$, 101 $[CH_3(CH_2)_4CH]$, and 83 $[CH_3(CH_2)_3CH{=}CH]$; CH_4 c.i.–m.s. of ethyl ester: m/z 301 ($M + 1$) and 283 ($M + 1 - H_2O$); m.s. of Me_3Si ether of ethyl ester: m/z 357 ($M - 15$), 327 ($M - 45$), 301 $[\overset{\overset{OSiMe_3}{|}}{C}H{-}(CH_2)_4COOC_2H_5]$, and 173 $[CH_3(CH_2)_4\overset{\overset{OSiMe_3}{|}}{C}H]$.

Methanolysis of the aqueous phase. — To a solution of the solid (145 mg) from the aqueous phase in absolute methanol (4.5 mL) was added 0.5% MeONa (0.5 mL). The mixture was kept for 100 min at room temperature, diluted with ice–water (5 mL), the base neutralized by addition of Amberlite IR-410 (CO_3^{2-}) resin, the methanol evaporated, and the aqueous phase extracted with chloroform (3 × 3 mL). The extracts were combined, washed with water, dried (Na_2SO_4), and evaporated. The residue was found to contain the methyl esters of C_{12}, C_{14}, C_{16}, and C_{18} acids by g.l.c. comparison with the mixture of methyl esters obtained by methanolysis of dichroside D_1.

Isolation and identification of sugars. — The aqueous fraction just obtained was evaporated to a syrup (105 mg) which was subjected to paper chromatography using 1-butanol saturated with water as the solvent, whereupon glucose, fucose, and rhamnose were detected. The syrup was chromatographed on a column of silica gel (10 g). Rhamnose (25 mg) and fucose (9 mg) were eluted in sequence with ethyl acetate saturated with water and glucose (8 mg) was eluted with acetone + 2% of water. Rhamnose, $[\alpha]_D$ +8.7° (*c* 1.0, H_2O); fucose, $[\alpha]_D$ +71° (*c* 1.0, H_2O); and glucose, $[\alpha]_D$ +46° (*c* 1.0, H_2O).

Acid hydrolysis of dichroside D_3. — Dichroside D_3 (10 mg) was heated with 90% aqueous HCO_2H (0.5 mL) on a boiling-water bath for 1 h, cooled, evaporated under diminished pressure, and the residue heated with 0.1M aq. H_2SO_4 (0.5 mL) for 10 h at 100°. The hydrolyzate was extracted with chloroform (3 × 0.5 mL), and the extracts were combined, washed with water, dried, and evaporated. To a solution of the residue (3 mg) in methanol was added an excess of an ethereal solution

of diazomethane, and the mixture was kept overnight at 0°, and then evaporated. The hydroxyaglycon was identified as the methyl ester of 11-hydroxyhexadecanoic acid by comparison with an authentic sample in g.l.c. In combined g.l.c. and CH_4 c.i.–m.s. it showed a peak at *m/z* 269 ($M - H_2O + 1$). The aqueous portion was made neutral with Amberlite IR-410 (CO_3^{2-}) resin, and evaporated. The residue was found to contain glucose, fucose, and rhamnose by comparison with authentic samples in p.c., and the ratios were established as 1:1:3 by l.c. using 17:3 acetonitrile/water as the solvent at a flow rate of 1 mL per min.

Mild hydrolysis of dichroside D_3 with acid. — Dichroside D_3 (5 mg) was kept with 1% of conc. HCl in 1,4-dioxane, and aliquots were withdrawn every 30 min, and applied to a strip of filter paper for chromatography. After applying four aliquots, the paper chromatogram was run for 16 h. The developed chromatogram showed that the aliquot drawn after 30 min contained glucose solely, whereas the subsequent aliquots contained other sugars as well.

Permethylation of dichroside D_3. — Sodium hydride (50% suspension in oil; 2 g) was placed in a round-bottomed flask fitted with a rubber septum. The hydride was washed with dry hexane (3 × 10 mL), and dried by flushing with dry N_2. Dry Me_2SO (20 mL) was added, and the flask flushed with N_2 *via* two injection-needles. The mixture was stirred for 4 h at 60° while N_2 was passed in; this gave a 2M solution of dimsyl sodium.

Dichroside D_3 (5 mg) was dissolved in dry Me_2SO (5 mL) in a 10-mL vial sealed with a rubber septum. Dimsyl sodium (2M; 2.5 mL) was added by means of a syringe while the mixture was stirred. It was then kept overnight at room temperature. The vial was cooled, methyl iodide (2.5 mL) was added dropwise, the mixture kept for 1 h at room temperature, the vial opened, and the methyl iodide evaporated by flushing with N_2. The mixture was then poured into water (5 mL), extracted with chloroform (4 × 5 mL), and the extracts combined, washed with water (3 × 5 mL), dried (Na_2SO_4), and evaporated. The residue was taken up in chloroform and purified by passage through a bed of silica gel. The product, a colorless, viscous mass, showed no hydroxyl band in the i.r. spectrum; e.i.–m.s.: *m/z* 915 ($M - C_{17}H_{33}O_3$), 741, 617, 567, 219, and 189; NH_3 e.i.–m.s.: *m/z* 1218 [$M + NH_4$]$^+$, 1030, 1000, 950, 932, 776, 758, 652, 602, 584, 478, 304, 286 (base peak, $C_{17}H_{34}O_3 + NH_4 - H_2O^+$), 236, and 206.

Preparation of hexitol acetates from the permethylated dichroside D_3. — Permethylated dichroside D_3 was heated with 90% aq. formic acid (3 mL) on a boiling-water bath for 1 h. The acid was removed by evaporation under diminished pressure, and the residue heated with 0.1M aq. sulfuric acid for 10 h at 100°. The hydrolyzate was made then neutral with Amberlite IR-410 (CO_3^{2-}) resin, filtered, concentrated to 2 mL, and sodium borohydride (30 mg) added. After 2 h, Amberlite IR-120 (H^+) resin was added to pH 3.5, and the mixture was filtered, evaporated, and traces of solvent co-distilled with methanol (3 × 5 mL).

The mixture of hexitols so obtained was treated with acetic anhydride (1 mL) and pyridine (1 mL) for 1 h at 100°, and the excess of reagents was removed by

TABLE IV

G.L.C.–M.S. AND G.L.C. ANALYSES OF HEXITOL ACETATES[a] OBTAINED FROM DICHROSIDES D_1, D_2, AND D_3

Hexitol acetates	*G.l.c.–m.s.*		*G.l.c.* R_T *(min)*[c]
	m/z	R_T *(min)*[b]	
1,5-Di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol	175, 161, 131, 117, 115, 101, 89	4.45	0.41
1,4,5-Tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol	203, 161, 143, 117, 101, 87	6.2	0.9
1,2,5-Tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol	233, 189, 173, 159, 131, 129, 115, 101, 99, 89, 87, 71	6.8	0.80
1,5-Di-*O*-acetyl-2,3,4,5-tetra-*O*-methylglucitol	205, 161, 145, 129, 117, 101, 87	7.2	1.00
1,3,4,5-Tetra-*O*-acetyl-2-*O*-methylrhamnitol	275, 215, 201, 173, 159, 141, 129, 117, 113, 99, 87, 58	7.9	1.23

[a]Partially methylated hexitol acetates were also prepared from dichrosides D_1 and D_2 by the procedure described for dichroside D_3, and were found to be the same as given in Table IV. [b]3% OV-1 column at 165°. [c]3% OV-225 column at 170°.

co-distillation with toluene. The residue, containing the hexitol acetates, was subjected to g.l.c. in a column (3% of OV-225) at 170°, and g.l.c.–m.s. using a g.l.c. column containing 3% of OV-1 at 160°. The results are summarized in Table IV.

Partial hydrolysis of dichroside D_2. — Dichroside D_2 (40 mg) was treated with 80% aq. formic acid (1 mL) for 2 h at 80°, cooled, and evaporated under diminished pressure. On t.l.c. in solvent *2*, the residue (35 mg) showed a spot for a new product having an R_F value slightly lower than that of dichroside D_2, along with two other spots corresponding to rhamnose and fucose. The presence of rhamnose and fucose was confirmed by paper-chromatographic comparison with authentic samples thereof.

The product was chromatographed on a column of silica gel (3.5 g), whereby the new product (8 mg) was obtained in pure form in the 10:2:1 chloroform–methanol–water eluate. It was subjected to methanolysis with MeONa by the procedure used for the methanolysis of dichroside D_1. The product, a trisaccharide, was a colorless, viscous mass.

Acid hydrolysis of the trisaccharide. — The trisaccharide (3 mg) was hydrolyzed by the procedure described for dichroside D_3. The hydrolyzate did not contain any fatty acid aglycon. Its p.c. showed the presence of glucose and rhamnose only, and their ratio was established as 1:2 by l.c. using, 17:3 acetonitrile–water as the solvent at a flow rate of 1 mL per min.

Preparation of hexitol acetates of the trisaccharide. — The trisaccharide was permethylated, the product hydrolysed, and the resulting partially methylated sugars were converted into their hexitol acetates according to the procedure described for dichroside D_3. The hexitol acetates obtained from dichroside D_4 were identified as 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol by g.l.c.–m.s. analysis.

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REFERENCES

1 C. R. SMITH, JR., L. H. NIECE, H. F. ZOBEL, AND I. A. WOLFF, *Phytochemistry*, 3 (1964) 289–299.
2 G. LEGLER, *Phytochemistry*, 4 (1965) 29–41.
3 H. C. SRIVASTAVA AND S. S. MISHRA, *J. Pharm. Sci.*, 56 (1967) 771–773.
4 S. N. KHANNA AND P. C. GUPTA, *Phytochemistry*, 6 (1967) 735–739.
5 H. OKABE AND T. KAWASAKI, *Tetrahedron Lett.*, (1970) 3123–3126.
6 H. WAGNER AND P. KAZMAIER, *Tetrahedron Lett.*, (1971) 3233–3236
7 J. P. S. SARIN, H. S. GARG, N. M. KHANNA, AND M. M. DHAR, *Phytochemistry*, 12 (1973) 2461–2468.
8 S. SINGH AND B. E. STACEY, *Phytochemistry*, 12 (1973) 1701–1705.
9 H. WAGNER AND G. SCHWARTING, *Phytochemistry*, 16 (1977) 715–717.
10 H. WAGNER, G WENZEL, AND V. M. CHARI, *Planta Med.*, 33 (1978) 144–151.
11 A. NIKOLIN, B. NIKOLIN, AND M. JANKOVIC, *Phytochemistry*, 17 (1978) 451–452.
12 G. ZEMPLÉN, *Ber.*, 59 (1926) 1254–1266; G. ZEMPLÉN AND D. KISS, *ibid.*, 60 (1927) 165–170
13 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
14 P. E. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG AND J. LONNGREN, *A Practical Guide to the Methylation Analysis of Carbohydrates, Chem. Commun., Univ. Stockholm*, 1976.
15 A. DE BRUYN, M. ANTEUNIS, R. DE GUSSEM, AND G. G. S. DUTTON, *Carbohydr. Res.*, 47 (1976) 158–163.