Carbohydrate Research, 212 (1991) 169–176
Elsevier Science Publishers B.V., Amsterdam

Structure of pescaproside E, a fatty acid glycoside from *Ipomoea pescaprae**

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(Received May 30th, 1990; accepted for publication October 29th, 1990)

ABSTRACT

Three fatty acid glycosides, designated pescaprosides A, B, and E, have been isolated from *Ipomoea pescaprae* (family Convolvulaceae). Chemical studies on pescaproside E, the major product, led to its characterisation as a pentaglycoside of 11-hydroxyhexadecanoic acid. Its sugar chain was esterified with 2-methylbutanoic and lauric acids. The structure of the pentaglycoside has been established as *O*-α-L-rhamnopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-fucopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→11)-oxyhexadecanoic acid.

INTRODUCTION

Numerous species of the genus *Ipomoea* (Convolvulaceae) are used in folk medicine all over the world[1–4]. Literature reports indicate that plants of this genus are a rich source of fatty acid glycosides[5,6]. Mono- and dihydroxy C_{14}, C_{15}, and C_{16} fatty acids are present as aglycons, and glucose, fucose, rhamnose, and quinovose constitute the sugar moieties. In a few cases the sugar chain is esterified with lower acids such as tiglic and 3-hydroxy-2-methylmethylbutanoic acid.

The unique structural features, of the convolvulaceous glycosides and their multiple pharmacological properties encouraged us to undertake a study of the extractives of *Ipomoea pescaprae*. Detailed chemical investigation resulted in the isolation of three fatty acid glycosides, pescaprosides A, B, and E. In the present paper the elucidation of the structure of pescaproside E is described.

RESULTS AND DISCUSSION

The ethanolic extract of the aerial parts of the dried, powdered plant was fractionated into hexane-, chloroform-, 1-butanol-, and water-soluble fractions. The hexane fraction was found to contain five fatty acid glycosides (t.l.c.), designated as pescaprosides A, B, C, D, and E. This fraction was therefore repeatedly chroma-

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0008-6215/91/$03.50

tographed, and pescaprosides A, B, and E were isolated. Since pescaproside E was the major component, it was taken up for structure elucidation.

Pescaproside E was obtained as an amorphous powder which showed a single spot on t.l.c. Because pescaproside E was obtained from the hexane fraction and was very nonpolar, it was considered to be profusely esterified. It was therefore subjected to alkaline methanolysis and the product was steam distilled. On mass spectral analysis, the distillate was found to contain the methyl ester of α-methylbutyric acid. The nonvolatile fraction was resolved into water- and chloroform-soluble fractions. G.l.c.–m.s. analysis of the chloroform-soluble fraction revealed the presence of the methyl ester of lauric acid.

The water-soluble fraction of the methanolysis product showed on t.l.c. (solvents *2,3*) two spots designated as pescaprosides E_1 and E_2 in decreasing order of their R_f values. These products were separated by column chromatography. Further treatment of pescaproside E_1 with 0.5M NaOH for 16 h at room temperature led to complete de-esterification, with the formation of pescaproside E_2 and lauric acid.

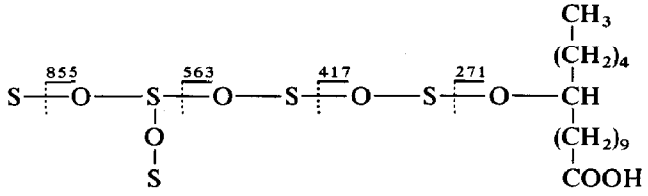
Pescaproside E_2 was subjected to acid hydrolysis with M aqueous sulphuric acid for 4 h at 100°. The hydrolysate was resolved into chloroform- and water-soluble portions. The chloroform-soluble portion was purified by chromatography, and the contained aglycon was identified as 11-hydroxyhexadecanoic acid by e.i.-m.s. of the methyl ester and of its trimethylsilyl ether.

Paper chromatography of the aqueous portion revealed the presence of rhamnose and fucose as the sugar moieties. The ratio Fuc:Rha was established as 1:4 by g.l.c. of the alditol acetates. The sugars were separated by column chromatography over silica gel and their specific rotations determined at equilibrium. The values were in complete agreement with those of L-rhamnose and D-fucose.

Structure of the sugar moiety. — Pescaproside E_2 was subjected to permethylation by the Hakomori method[7], followed by acid hydrolysis. The resulting partially methylated sugars were converted into their alditol acetates by reduction with sodium borohydride followed by acetylation. Finally, the mixture of alditol acetates was analysed by g.l.c.–m.s. and g.l.c. according to the method of Lönngren *et al.*[8]. The components were identified as 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylfucitol, and 1,3,4,5-tetra-*O*-acetyl-2-*O*-methylrhamnitol. Formation of the foregoing methylated alditol acetates, and the negative f.a.b.–m.s. fragmentation of pescaproside E_2, clearly indicated that the sugar chain consisted of two terminal rhamnose units joined to the end unit of a sugar chain comprised of two rhamnosyl and one fucosyl residues, as shown in formula **1**. However, in the above chain the fucosyl residue could occupy either the reducing-end position, joined to the aglycon, or the penultimate (2) position.

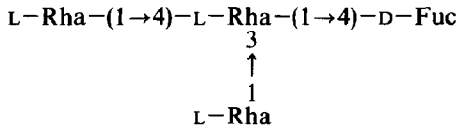
In order to settle this issue selective cleavage of the sugar chain was tried by treatment of pescaproside E, E_1, and E_2 with formic acid and trifluoroacetic acid under varying regimes of acid concentration, temperature, and time. Satisfactory results were achieved only by the hydrolysis of pescaproside E_1 with trifluoroacetic acid for 16 h at room temperature. This led to the appearance on t.l.c. of three major spots, designated

as compounds E_3, E_4, and E_5. These products were separated by column chromatography. Each of them was separately permethylated by the Hakomori method[7], and acid-hydrolysed.



M^-, m/z 1001 (S = deoxyhexosyl unit)

1



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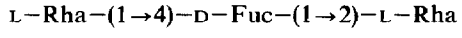

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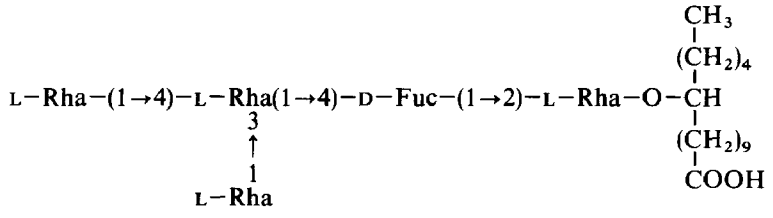

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The partially methylated sugars so formed were then converted to their alditol acetates, and the mixtures analysed by g.l.c.–m.s. The alditol acetates derived from compounds E_3 and E_5 were identified as 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylfucitol, and 1,3,4,5-tetra-*O*-acetyl-2-*O*-methylrhamnitol. Consequently the structure of the sugar chain of both E_3 and E_5 could be represented as **2**. Evidently, both compounds E_3 and E_5 contained the same tetrasaccharide unit, and the difference in their polarities (t.l.c.) was due to differences among the fatty acyl groups attached to their nonanomeric hydroxyl groups. These ester groups were obviously cleaved during the permethylation of the compounds.

The alditol acetates derived from compound E_4 were identified as 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylfucitol, and 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol. Thus, the formation of an alditol acetate

corresponding to a terminal rhamnosyl unit, without the formation of one corresponding to the disubstituted rhamnosyl residue, clearly suggested that the terminal sugar unit of compound E_4 must have arisen from the disubstituted rhamnosyl unit of pescaproside E_1. Compound E_4 could therefore be represented as **3**. Then, the rhamnose unit giving rise to 3,4-di-*O*-methylrhamnose from pescaproside E_2 and compound E_4 must be occupying the reducing end of the oligosaccharide, with its anomeric carbon in glycosidic alliance with the 11-hydroxyl group of the aglycon. The formation of compounds E_3, E_4, and E_5 from pescaproside E_1 clearly indicated the total sequence of sugars in the latter, as shown in **4**.

Further, the conformations of the sugar residues and the configurations of their linkages were established by n.m.r. studies on pescaproside E_2. Thus in the 400 MHz ^{1}H-n.m.r. spectrum of pescaproside E_2 four signals for anomeric protons of rhamnosyl units were observed at δ 5.18, 5.06, 4.99, and 4.91 ($J_{H\text{-}1,H\text{-}2}$ 2 Hz for all). By specific C-H decoupling experiments the ^{1}H signals were correlated with ^{13}C signals located at δ 102.48, 102. 48, 102.15, and 101.15 respectively. The $J_{C\text{-}1,H\text{-}1}$ values of these signals were found to be 169, 169, 172, and 172 Hz, respectively, by the application of DANTE technique. These $J_{C\text{-}1,H\text{-}1}$ and $J_{H\text{-}1,H\text{-}2}$ values are consistent with α-linkages of L-rhamnopyranosyl units in the $^1C_4$ conformation[9,10]. The remaining anomeric proton and carbon signals*, at δ 4.48 and δ 100.65 respectively, belong to the fucosyl unit. The $J_{H\text{-}1,H\text{-}2}$ (7 Hz) and $J_{C\text{-}1,H\text{-}1}$ (163 Hz) values indicate that the D-fucosyl residue exists in $^4C_1$ pyranose form and is coupled to the next rhamnopyranosyl unit through a β linkage. The structure of pescaproside E_2 is thus firmly established as **5**, and pescaproside E is the derivative having α-methylbutyric and lauric acids esterified to its nonanomeric hydroxyl groups.

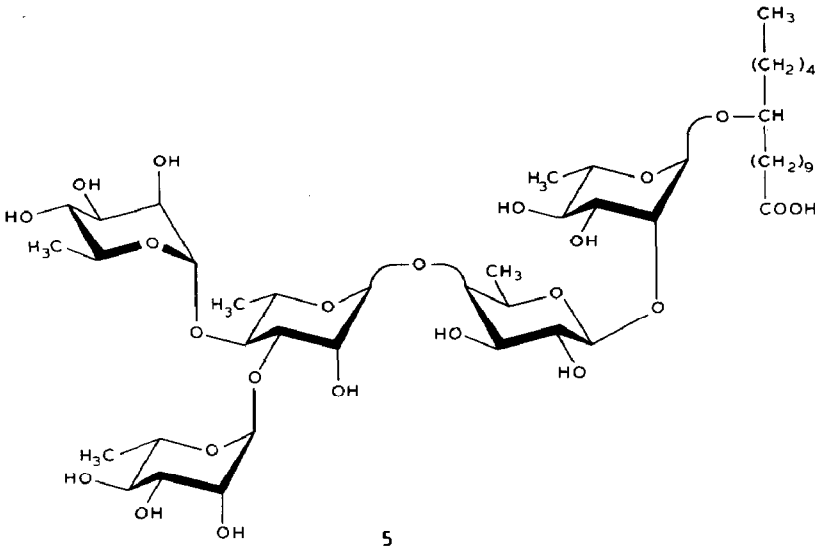
TABLE I

G.l.c.–m.s. and g.l.c. analyses of alditol acetates obtained from pescaprosides E, E_1 and E_2

Alditol acetates	*G.l.c.–m.s.*	*G.l.c.*	
	M.s. (m/z)[a]	t_R (*min*)[b]	t_R (*min*)[c]
1,5-Di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol	175, 161, 131, 117, 115, 101, 89	8.2	0.38
1,4,5-Tri-*O*-acetyl-2,3-di-*O*-methylfucitol	247, 203, 161, 143, 117, 101	10.1	1.08[d]
1,2,5-Tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol	233, 181, 175, 173, 159, 131, 129, 117, 115, 101, 99, 89, 87	10.5	0.86
1,3,4,5-Tetra-*O*-acetyl-2-*O*-methylrhamnitol	275, 215, 201, 173, 159, 141, 129, 117, 113, 99	11.3	1.37

[a] At 20 eV. [b] SE-30 column, from 160 to 220° at 10° per min. [c] OV-225 (3%) column at 170°. [d] Not given in the literature.

* Attention is drawn to the chemical shifts of these signals, which are at somewhat higher field than expected. This appears to be due to the anisotropic effect of some neighbouring group, also evident in the case of the fucosyl anomeric H of dichroside D_2 (ref. 6).



5

EXPERIMENTAL

General. — I.r. spectra were recorded on a Perkin–Elmer Infracord 177 or a Beckman Ac-1 instrument in KBr, or chloroform. ^{1}H-n.m.r. spectra were recorded at 90 MHz using a Perkin–Elmer R-32 or at 400 MHz a Bruker WM-400 n.m.r. spectrometer (^{13}C-n.m.r. at 100.13 MHz), in $CDCl_3$ or D_2O using tetramethylsilane and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as internal standards, respectively. Mass spectra were recorded with a Jeol JMS-D300 mass spectrometer, and g.l.c.–m.s. was performed using the same instrument coupled with a Chemito 3800 gas chromatograph. Negative f.a.b. mass spectra were obtained on a MS 9/50 TC instrument, using a copper probe tip to introduce the compound dissolved in a glycerol matrix. Xenon atoms of 6 kV average energy were used for bombardment. Optical rotations were measured in a 1 dm tube with a Jasco-Dip 180 or Perkin–Elmer 241 polarimeter. T.l.c. analysis was effected on silica gel G using the following solvents: (*1*), 8:1:1 ethyl acetate–methanol–water; (*2*) 8:8:1 ethylacetate–acetone–water; (*3*), 12:16:3:0.02 ethyl acetate–acetone–water–acetic acid; (*4*), 17:3 chloroform–methanol. Paper chromatography was carried out on Whatman No.1 filter paper using 1-butanol saturated with water as the eluent.

Extraction and isolation of pescaprosides A, B, and E. — The air-dried aerial parts (24 kg) of *Ipomoea pescaprae* collected in Konark, Orrisa was extracted with ethanol (3 × 40 L) and the ethanolic extract concentrated under reduced pressure at 50°. The concentrate was extracted with *n*-hexane (4 × 2 L), chloroform (4 × 2 L), and 1-butanol (4 × 2 L). Evaporation of the respective extracts afforded hexane- (550 g), chloroform- (100 g), 1-butanol- (120 g), and water-soluble (100 g) fractions. The hexane-soluble fraction showed five spots for glycosides on t.l.c. in solvents *1* and *4*. They were designated pescaprosides A, B, C, D, and E in decreasing order of their R_F values. A portion (70 g) of the *n*-hexane soluble fraction was subjected to coarse separation on a column of silica gel (1 kg). Fractions 15–30, eluted with 2:1 ethyl acetate–acetone,

afforded the glycoside pescaproside E with other minor compounds, which were removed by rechromatography in the same system to give pure pescaproside E (5 g). Fractions 10–12, eluted with 7:3 ethyl acetate–acetone, gave a mixture of pescaproside A and B. This mixture was rechromatographed on silica gel using 19:1 chloroform–methanol. Fifty fractions were collected (250 mL each). T.l.c. of fractions 32–37 in solvent system *5* revealed the presence of pescaproside A with slight streaking, which was removed by p.l.c. of the material in the same solvent system. Pescaproside E was obtained from fractions 40–45 and was purified by p.l.c.

Methanolysis of pescaproside E. — To a solution of pescaproside E (1.5 g) in absolute methanol (45 mL) was added 0.5% methanolic MeONa (5 mL), and the mixture was kept for 16 h at room temperature. The base was then neutralised with Amberlite IR-120 (H^+) resin, and the neutral solution was steam distilled. The mass spectrum of the distillate was recorded by placing it in a capillary tube which was then inserted into the direct inlet system of the spectrometer. The distilled component was identified as methyl α-methylbutyrate acid, e.i.-m.s.: *m/z* 101 $(M - 15)^+$, 88 $[CH_3CH=C(^+OH)OCH_3]$, 87, and 85; c.i.-m.s. (NH_3): *m/z* 134 $(M + NH_4)^+$ and 117 $(M + H)^+$.

The nonvolatile portion was partitioned between chloroform and water. The chloroform fraction was subjected to g.l.c.–m.s. analysis on a 3% OV-1 column at 225°. This analysis showed a single g.l.c. peak for methyl laurate, t_R 2.3 min, M^+ at *m/z* 214.

The water-soluble portion was evaporated to dryness. The residue (400 mg) showed two spots on t.l.c. (solvent *2*), designated as pescaprosides E_1 and E_2. These pescaprosides were purified by column chromatography over silica gel (35 g). Fifty fractions (25 mL each) were collected. Fractions 10–20, eluted with 1:1 water-saturated ethyl acetate–acetone were evaporated to give pescaproside E_1 as a colourless, amorphous solid (280 mg). Evaporation of fractions 30–40, eluted with 49:1 acetone–water, furnished pescaproside E_2 (90 mg).

Pescaproside E_1 (150 mg) was treated with 0.5M aqueous NaOH (3 mL) for 16 h at room temperature. The base was neutralised by the addition of Amberlite IRA-410 (CO_3^{2-}) resin. The solution was extracted with chloroform (5 × 3 mL), and then evaporated. The residue containing pescaproside E_2 (120 mg) on column chromatography over silica gel (12 g), using 49:1 acetone–water as eluent, gave pure pescaproside E_2 (110 mg) as a colourless, amorphous powder, $[\alpha]_D$ +69.3 (*c* 1.0, H_2O); negative f.a.b.–m.s.: *m/z* 1001 $(M - H)^-$, 855 (1001 − 146, m* at 730.5), 563 (855 − 292), 417 (563 − 146, m* at 309), 271 (417 − 146, m* at 176.5, aglycon).

Acid hydrolysis of pescaproside E_2. — Pescaproside E_2 (150 mg) was heated with 90% aqueous formic acid for 1 h at 100°. The solution was cooled and evaporated under diminished pressure, and the residue heated with 2M aqueous H_2SO_4 (1 mL) for 2 h at 100°. The hydrolysate was then extracted with chloroform. The organic layer was washed with water, dried over anhydrous sodium sulphate, and evaporated to give crude aglycon (30 mg). The aglycon was converted into its methyl ester by treatment with diazomethane, and the crude methyl ester was purified by chromatography on silica gel, using hexane–acetone as the eluent. Ten fractions of 10 mL each were

collected. Fractions 5–8 contained pure methyl 11-hydroxyhexadecanoate, showing a single peak by g.l.c. on a column of 3% SE-30 at 250°; i.r.: $\nu_{max}^{CHCl_3}$ 3300, 2860, 1720, 1450, and 1175 cm^{-1}; ^1H n.m.r. ($CDCl_3$): δ 0.88 (t, 3 H, CH_3), 1.28 (br. s, 29 H, CH_2 and CH_3), 2.29 (t, 2 H, J 7 Hz, $COCH_2$), 3.65 (s, 3 H, OCH_3); e.i.-m.s.: m/z 286 $(M)^+$, 268 $(M - H_2O)^+$, 199 $(M-87)^+$, 101 $[CH_3(CH_2)_4CHOH]^+$, and 83 $[CH_3(CH_2)_3\ CH=CH]^+$; c.i.-m.s. of methyl ester (CH_4): m/z 287 $(M + 1)^+$, and 269 $(M + 1 - H_2O)^+$; e.i.-m.s. of Me_3Si ether of methyl ester: m/z 343 $(M - 15)^+$, 328 $(M - 30)^+$, 287 $[Me_3SiOCH(CH_2)_9\ COOCH_3]^+$, and 173 $[CH_3(CH_2)_4\ CHOSiMe_3]^+$.

Isolation and identification of sugars. — The aqueous portion was neutralised with IRA-410 (CO_3^{2-}) resin and evaporated to a syrup (100 mg). It was found to contain rhamnose and fucose on paper chromatography with the authentic samples. The ratio of these sugars was established as rhamnose:fucose 4:1 by g.l.c. analysis of their alditol acetates, obtained by treatment of the mixture (10 mg) with sodium borohydride in water and acetylating the product with acetic anhydride in pyridine. The sugars were also separated by chromatography of the syrup (90 mg) on a column of silica gel (10 g). Rhamnose (46 mg) and fucose (15 mg) were eluted in sequence with ethyl acetate saturated with water. Rhamnose had $[\alpha]_D$ +9.2° (equilibrium, c 1.0, H_2O), and fucose had $[\alpha]_D$ + 72° (equilibrium, c 1.0, H_2O).

Methylation and preparation of alditol acetates[8] from pescaproside E_2. — Pescaproside E_2 (10 mg) was per-*O*-methylated with sodium methylsulfinylmethanide (2M, 5 mL) and methyl iodide (10 mL). The product (11 mg) was chromatographed over silica gel (2 g) using *n*-hexane–acetone as the solvent. The fraction eluting with a 9:1 solvent mixture yielded pure per-*O*-methylated product as a colourless, viscous mass devoid of any hydroxyl band in the i.r. spectrum.

Methylated pecaproside E_2 (7 mg) was hydrolysed with 90% aqueous formic acid and the product reduced and acetylated. The residue containing the alditol acetates was subjected to g.l.c. on a column of 3% OV-225 at 170°, and g.l.c.–m.s. using a column containing 3% SE-30. The column was programmed from 160° to 220° at the rate of 10° per min. The results are summarised in Table I.

Selective cleavage of pescaproside E_1. — Pescaproside E_1 (100 mg) was treated with trifluoroacetic acid for 16 h at room temperature and the solution was evaporated under diminished pressure. On t.l.c. in solvent *3* the residue (90 mg) showed three major spots, designated as compounds E_3, E_4, and E_5. These were separated by column chromatography over silica gel (10 g), using mixtures of ethyl acetate saturated with water with increasing proportions of acetone as eluents.

ACKNOWLEDGMENTS

The authors are grateful to Dr. F. A. Mellon, AFRC Institute of Food Research, Norwich, U.K., for running the f.a.b. mass spectra of the pescaprosides. They are also thankful to the staff of the Regional Sophisticated Instruments Centre, CDRI, for spectral and analytical data, to Dr. B. N. Mehrotra for identifying and supplying the plant material, and to Mr. J. P. Chaturvedi for technical assistance.

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