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THE STRUCTURES OF FIBRAURIN AND A MINOR PRODUCT FROM *FIBRAUREA CHLOROLEUCA*

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Abstract—Fibraurin, a diterpenoid extracted from *Fibraurea chloroleuca*, has been shown to have structure **1**; a minor product from the same extract has been identified as 6-hydroxyfibraurin (**16**).

THE bark and stems of *Fibraurea chloroleuca* Miers (*Menispermaceae*) collected in Singapore[1] gave a crystalline bitter principle which we have named fibraurin (**1**).

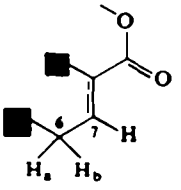
FIG. 1.[2] Gross physical properties of fibraurin, and data pertaining to the two lactones.

Fibraurin, $C_{20}H_{28}O_7$, m.p. 288–289°
$[\alpha]_D$ − 28° (*c* 1·04 in pyridine), no Cotton effect in RD curve above 250 mμ
UV (MeOH): 211 (9,300), 230 mμ (*ε* 9,000)
IR (KBr): 3460 (OH), 1766 (C–O), 1690 (C=O), 1632 cm^{-1} (C–C); and furan bands at 3120, 1600, 1505, 1021, 874, 815 cm^{-1}
NMR (DMSO, Fig. 2): two t-Me groups at 1·06 and 1·15 ppm

The lactone groups in fibraurin
Potentiometric titration: 1·12 moles of alkali uptake.
Saturated lactone: 1766 cm^{-1} (KBr), not cleaved in warm ethanolic alkali.
UV (MeOH) of tetrahydro deriv. **2** at 231 mμ (6,500) disappears in hexahydro deriv. **3**

NMR (DMSO, Fig. 2):

H_7 7·25	J	6a, 7 3·0 c/s
H_{6a} 1·7		6b, 7 8·0
H_{6b} 2·3		6a, 6b 15·5



Microanalyses and high resolution MS established its molecular formula of $C_{20}H_{28}O_7$, while the UV (211 mμ), IR (Fig. 1) and NMR spectra (Fig. 2) suggested that a β-substituted furan ring was present. Of the seven oxygen atoms, eventually four could be ascribed to two lactone groups, but this was not at all obvious from the titration, since, warming a solution of fibraurin in 0·1N 50% ethanolic NaOH for 1 hr and back titration with hydrochloric acid indicated that only 1·12 moles of alkali had been consumed. When this back-titrated neutral solution was evaporated to dryness, and the residual sample and sodium chloride mixture was made into a KBr disk, the IR spectrum still retained the 1766 cm^{-1} band (Fig. 1), but the 1690 cm^{-1}

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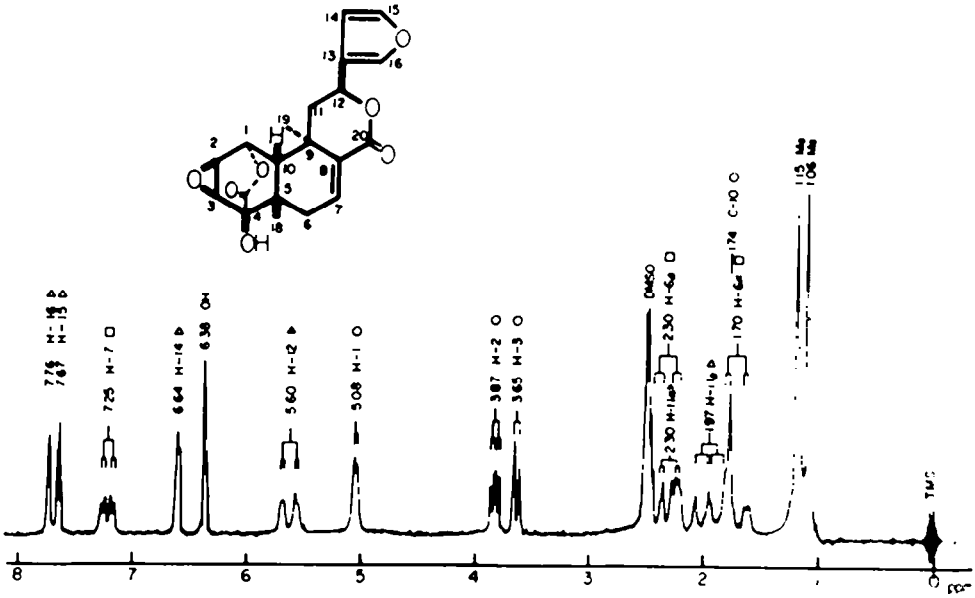
[1] K. Nakanishi, S. Sasaki, A. K. Kiang, J. Goh, H. Kakisawa, M. Ohashi, M. Goto, J. Watanabe, H. Yokotani, C. Matsumura and M. Togashi, *Chem. Pharm. Bull.* 13, 882 (1965).
[2] Black squares in partial structures denote fully substituted carbon atoms.

FIG. 2. NMR spectrum of fibraurin in DMSO-d_6, 100 Mc, ppm from internal TMS

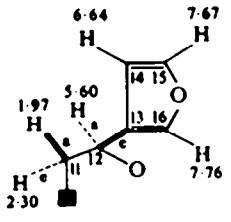


The squares, triangles and circles in the figure denote the coupled proton systems shown in Figs 1, 3, and 4, respectively.

band (νC=O) had now been replaced by carboxylate bands and the intensity of the 1632 cm^{-1} band (νC=C) was greatly reduced. However, since fibraurin lacks a ketone group, as suggested by the absence of a Cotton effect in its RD curve above 250 mμ, the 1766 cm^{-1} absorption could only be assigned to a lactone group.

The unsaturated lactone group which is responsible for the 231 mμ absorption in tetrahydrofibraurin (**2**) (furan ring reduced to tetrahydrofuran) is of the cisoid type because of the strong intensity of the IR 1632 cm^{-1} band and the low NMR chemical shift of the olefinic proton. NMR further established that this lactone group was present in the moiety shown in Fig. 1.

FIG. 3.[a] Chemical shifts and coupling constants of furan moiety (see Fig. 2).



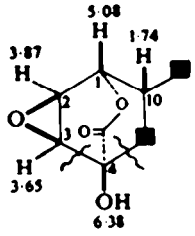
J			14 c/s
11α,	11β		
11β,	12		12
11α,	12		2·5
14,	15		1·8
15,	16		1·8
14,	16		0·8
12,	14		<0·5*
12,	15		<0·5*
12,	16		<0·5*

* small coupling established by decoupling experiments.

Presence of the furan ring was suggested from the UV spectra of fibraurin, the hemiacetal **4b** (λ_{max}^{MeOH} at 211 mμ with ε 3,900) and the deoxo derivative **5** (λ_{max}^{MeOH} at 204 mμ with ε 3,900). The NMR spectrum of fibraurin not only confirmed the presence of a β-substituted furan ring but also provided information on the nature of this substituent. Decoupling experiments at 100 Mc disclosed that H-12 (a quartet at

5·60 ppm; the X of an ABX) is (i) α to an oxygen function, (ii) adjacent to a methylene group (two quartets; the AB of an ABX not further coupled), and moreover (iii) weakly coupled (Js <0·5 c/s) to the three furan protons, thus establishing the moiety shown in Fig. 2. The 12 c/s coupling constant indicates an axial-axial relationship between H-11β and H-12.

FIG. 4.[8] Chemical shifts and coupling constants of the lactone–epoxide moiety.



J 1, 2	2·9 c/s
1, 3	0·5
1, 10	<0·5*
2, 3	4·2

* small coupling established by decoupling experiments. Wavy lines indicate bonds not necessarily derivable from the evidence given so far.

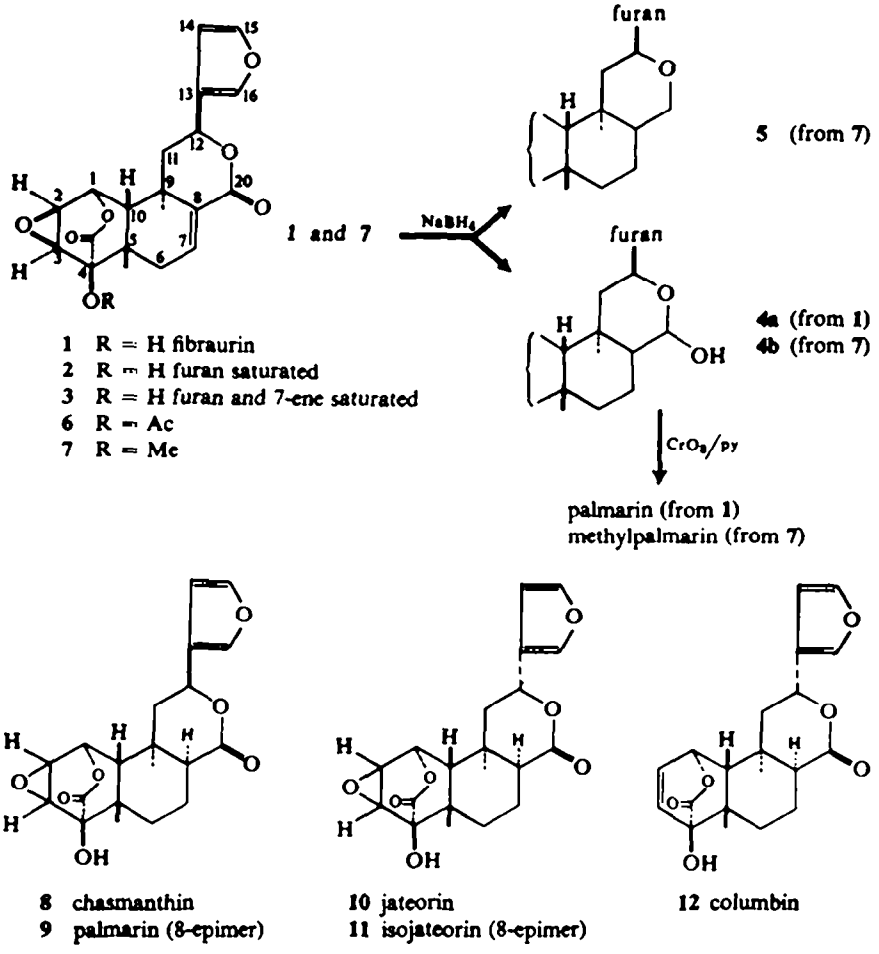
Since the NMR of fibraurin showed a one-proton signal at 6·38 ppm in DMSO-d_6, which disappeared upon addition of D_2O, it was clear that a tertiary OH group was present; this was confirmed by the NMR of fibraurin acetate (**6**). Moreover, the fact that fibraurin readily furnished a methyl ether **7** upon treatment with dimethyl sulfate suggested that the OH group was attached to an electron-withdrawing function. The evidence presented so far has clarified the nature of 6 of the 7 oxygen atoms. Now there are still *two* low-field NMR signals at 3·87 and 3·65 ppm respectively (Fig. 2) to be explained, and this together with the fact that one oxygen also remains unaccounted for leads to consideration of an epoxide function. This assumption is supported by the observation of a 1,3-coupling[5] as well as a 1,2-coupling between the two epoxide protons and the neighbouring proton at the low-field of 5·08 ppm (Fig. 4). The 5·08 ppm signal was weakly coupled to still another singlet at 1·74 ppm. The data summarized in this paragraph enabled us to derive the partial structure shown in Fig. 4, although the resistance of fibraurin to mild acid treatment (e.g., boiling in BF_3-etherate and benzene) was rather unexpected.

As described already (Fig. 1) fibraurin contains two tertiary Me groups, and the functions derived above indicate that it should have a bicyclic carbon skeleton. Furthermore, the unsaturated lactone shown in Fig. 1 was most probably 6-membered because the IR of hexahydrofibraurin (**3**) absorbed at 1730 cm^{-1} (KBr). These facts in conjunction with the partial structures derived in Figs. 1, 3 and 4 lead to structure **1** (Fig. 5) for fibraurin, which is also consistent with biogenetic considerations.

Structure **1** simply corresponded to the bis-dehydro derivative of palmarin (**9**) and its congeners isolated from Colombo roots[4–6] and to which structures **8–11** had been assigned.[6–7] Fortunately, a direct correlation between fibraurin and palmarin

[5] D. D. Elleman, S. L. Manatt and C. D. Pearce, *J. Chem. Phys.* **42**, 650 (1965).
[4] F. Wessely and K. Schönol, *Monatsh.* **71**, 10 (1938).
[5] K. Feist and W. Volksen, *Liebigs Ann.* **534**, 41 (1938).
[6] D. H. R. Barton, K. H. Overton and A. Wylie, *J. Chem. Soc.* 4809 (1962).
[7] S. K. Balasubramanian, D. H. R. Barton and L. M. Jackson, *J. Chem. Soc.* 4816 (1962).

FIG. 5



could be readily accomplished by the following three routes:

(i) hexahydrofibraurin (3), which was prepared by reduction of fibraurin with Pd–C–H_2O and then with PtO_2–EtOH, was identical[8] with tetrahydropalmarin;

(ii) chromic acid oxidation of the hemiacetal **4b** derived from methylfibraurin (Fig. 5) afforded a lactone that was identical[8] with methylpalmarin;

(iii) finally, sodium borohydride reduction of fibraurin (1) followed by oxidation of the hemiacetal (Fig. 5) gave palmarin itself.[8]

Conversion of isojateorin (11) and isocolumbin[9] (epimeric at C-8 in 12) into a common derivative has proven[7] that isojateorin is the 1,2-epoxide of isocolumbin; on the other hand, stereostructure 12 has been forwarded for columbin.[10] Since the relation between isojateorin and palmarin is known,[6] and that between fibraurin and palmarin has also been made clear, fibraurin can be expressed by structure 1, excepting

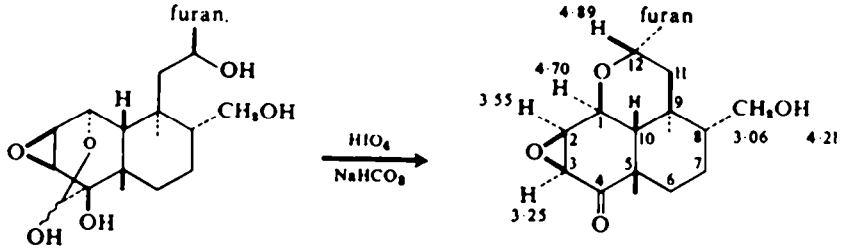
[8] Checked by $[\alpha]_D$, m.p., IR and TLC.

[9] D. H. R. Barton and D. Elad, *J. Chem. Soc.* 2085 (1956).

[10] K. H. Overton, N. G. Weir and A. Wylie, *Proc. Chem. Soc.* 212 (1961).

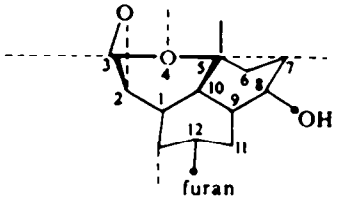
the stereochemistry of the 2,3-epoxide ring. For this we propose a β-configuration based on the following evidence.

FIG. 6. Configuration of the 2,3-epoxide ring.



13

14 NMR in DMSO-d_6
J 1, 2 2·2 c/s
2, 3 4·2
1, 10 8·3
11, 12 11·0, 3·0
OH 4·5 (triplet)



15 Octant projection of epoxy ketone **14**

LAH reduction[6] of palmarin, which in turn was derived from fibraurin (Fig. 5), gave the hemiacetal **13** as an oil;[6] oxidation of **13** with periodic acid at pH 7[6] yielded the crystalline monohydroxy ketone **14**, the structure of which is based on NMR evidence. Although this reaction sequence was carried out by following the conditions employed by Barton *et al.*[6] on palmarin, the product was **14** (ether ring formed by nucleophilic displacement) instead of the "trihydroxy ketone" formed by simple cleavage of the α-glycol system in **13**. The ketone **14** exhibited a negative Cotton effect in the RD curve, which in view of the findings[11] that epoxide rings make a rotatory contribution opposite to those of alkyl groups in terms of the octant rule, indicates that the epoxide ring is β-oriented (see octant diagram **15**).

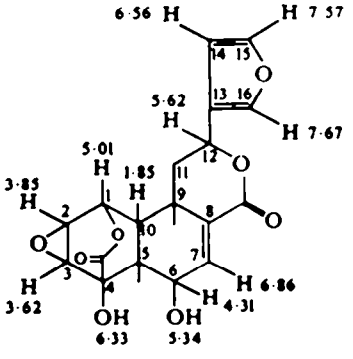


FIG. 7

m.p. 303–304° $[\alpha]_D^{29}$ +23·6° (*c* 1·13 in pyridine)
IR (KBr): 3500, 3400, 1773, 1717, 1644 cm^{-1}
UV (MeOH): 209 mμ (ε 15,000)
230 mμ (shoulder, ε 10,000)
NMR (DMSO-d_6 + 30% CCl_4, 100 Mc)
Me: 1·15 (3H, s) and 1·19 (3H, s)
H-11: 1·90 (1H, q, J 13·5 and 12) and
2·30 (1H, q, J 13·5 and 2·5)

J	1, 2	2·9 c/s	13, 14	1·8 c/s
	1, 3	0·5	14, 15	1·8
	1, 10	<0·5	14, 16	0·8
	2, 3	4·2	15, 16	0·8
	6, 7	2·8	6, OH	2·8

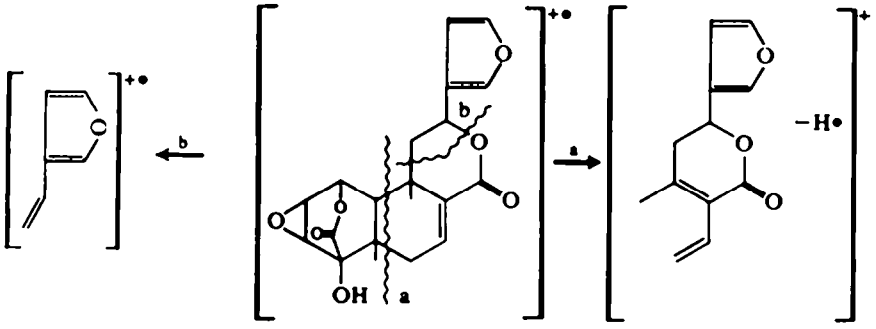
[11] C. Djerassi, W. Klyne, T. Norin, G. Ohloff and E. Klein, *Tetrahedron* **21**, 163 (1965).

Finally, two other minor products accompanied the isolation of fibraurin. One was identified as chasmanthin (**8**) (identical m.p. and IR, and conversion to palmarin); the other minor product is assigned a structure corresponding to 6-hydroxyfibraurin (**16**) from the physical data shown in Fig. 7. Although there were indications of intramolecular hydrogen-bond formation between the two OH groups (IR), the configuration at C-6 could not be determined from this evidence or from the NMR coupling constants because of the flexibility in the conformations of both ring B and the unsaturated lactone ring.

Attempts to convert fibraurin into the minor product by treatment with SeO_2–Ac_2O–AcOH, $Hg(OAc)_2$–AcOH, Pb_3O_4–AcOH, or CrO_3–AcOH unfortunately did not give any well characterized product.

The NMR data given in Fig. 7 is in complete agreement with the structure proposed; however the OH signals are anomalous and cannot be explained so far. The 6-OH appears as a pair of doublets at 5·31 and 5·37 (both with 3 c/s splittings); the 4-OH shows as a pair of singlets at 6·31 and 6·35 ppm. Decoupling experiments confirm that the C_6-proton is coupled to the 6-OH. Moreover, irradiation of the water signal at 3·14 ppm causes collapse of both sets of OH signals thus indicating that significant proton exchange is occurring. Addition of D_2O caused the slow disappearance of all OH signals.

FIG. 8



C_5H_6O calcd. 94·042 obsd. 94·040 | $C_{20}H_{20}O_7$ calcd. 372·121 obsd. 372·123 | $C_{13}H_{11}O_3$ calcd. 203·071 obsd. 203·071

Corresponding peaks in tetrahydrofibraurin (**2**):

$C_5H_{10}O$ calcd. 98·073 obsd. 98·073 | M^+ peak not detected | $C_{13}H_{11}O_3$ calcd. 207·102 obsd. 207·105

Finally, the high resolution MS (direct inlet) of fibraurin (**1**) and tetrahydrofibraurin (**2**) are briefly described. As already mentioned, fibraurin gives an M^+ peak corresponding to $C_{20}H_{20}O_7$ when subjected to electron impact (30 eV, accelerating voltage 37·5 KV, $3·5 \times 10^{-6}$ mm Hg); under the same conditions, the tetrahydro derivative gives no M^+ peak, the highest peak being at $C_{19}H_{23}O_6$ (loss of CHO). Two abundant ions are observed at *m/e* 203 and 94, which are due to the retro-Diels-Alder fragmentation (a) and allylic cleavage (b), respectively. The assignment is supported by the fact that these peaks are shifted four mass units higher in the spectrum of tetrahydrofibraurin. Fragmentations pertaining to ring A will be discussed in a separate paper.

EXPERIMENTAL

The following instruments were used for measuring the physical data: UV, Hitachi Perkin–Elmer Model 139; IR, Hitachi Model EPI-2; NMR, Varian A-60 and HA-100; MS, Hitachi RMU-5B and JEOL JMS-01 High Resolution Instrument; RD, JASCO Model ORD/UV 5; $[\alpha]_D$ Rudolf polarimeter.

NMR spectra were assigned with the aid of decoupling at 100 Mc and by measuring each compound, where necessary, in a variety of solvents and solvent mixtures ($CDCl_3$, benzene, pyridine, DMSO-d_6, etc) to resolve overlapping multiplets. The following abbreviations have been used in the text: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. Chemical shifts are expressed in ppm from internal TMS.

Parent peaks in the MS are indicated by M^+; values expressed in round figures were measured on the Hitachi RMU-5B, whereas values expressed to three places of decimals were measured on the JEOL JMS-01.

Isolation of fibraurin (**1**) *from the bark.* The powdered bark (1·5 Kg) of *Fibraurea chloroleuca* Miers was extracted with boiling MeOH and the extracts were concentrated *in vacuo*. The residual brown oil and crystals were washed with ether and purified by recrystallization from MeOH. The crude crystals gave 5·2 g fibraurin as pale yellow crystals; yield from dried bark, 0·34%. (Found: C, 64·50; H, 5·32; M^+ 372·118. $C_{20}H_{28}O_7$ requires: C, 64·51; H, 5·41%; MW 372·121.)

Isolation of chasmanthin (**8**), *fibraurin* (**1**) *and minor product* **16** *from the stems.* The pulverized stems (11 Kg) of *Fibraurea chloroleuca* Miers were extracted continuously with ether. The ether extract was concentrated to dryness and the residue washed with ether. A soln of the residual crude crystals in MeOH was absorbed on Celite and the Celite placed on top of a column of silica gel which was then eluted with a $CHCl_3$–EtOH mixture by gradually changing the solvent composition from 100:1 to 50:1. Elution afforded 58 mg of chasmanthin (5×10^{-4}%), 5 g of fibraurin (0·045%) and 25 mg of the minor product **16** ($2·5 \times 10^{-4}$%) in that order.

Titration of fibraurin (**1**). Fibraurin (53·2 mg) was dissolved in 5 ml of 0·1N 50% ethanolic NaOH, the soln was heated on the steam-bath for 1 hr and then back-titrated with 0·1N HCl; the uptake of alkali was 1·12 moles. After neutralization, the soln was dried *in vacuo* and the residual mixture of the sample and NaCl was made into a KBr disk. IR (KBr) 1760 (lactone), 1642 (C=C), 1560, 1400 cm^{-1} (COONa).

Fibraurin acetate (**6**). Fibraurin (100 mg) was acetylated by heating for 3 hr in 6 ml Ac_2O and 2 g AcONa to give 80 mg of its acetate as needles, m.p. 315–317° (MeOH). (Found: C, 63·79; H, 5·22. $C_{26}H_{30}O_8$ requires: C, 63·76; H, 5·35%.) UV (MeOH): 211 (9,300), 230 mμ (ε 9,000). IR (KBr): 1780, 1753, 1690, 1635 cm^{-1}. NMR (CF_3COOH): 2·43 ppm (3H, s, OAc).

Methylfibraurin (**7**). Fibraurin (100 mg) was dissolved in 5% aqueous alkali by heating on a steam bath, and the soln was treated at room temp with Me_2SO_4 (2 ml) and then with 10% NaOH aq. Acidification with dil. HCl aq, afforded crystals (90 mg) which, after recrystallization from MeOH, had m.p. 268–269°. (Found: C, 65·44; H, 5·80. $C_{21}H_{28}O_7$ requires: C, 65·27; H, 5·74.) IR($CHCl_3$): 1780, 1713, 1638 cm^{-1}. NMR (DMSO-d_6): 3·57 ppm (3H, s, OMe).

Sodium borohydride reduction of methyl fibraurin (**7**) *to the hemiacetal* **4b** *and the deoxo derivative* **5**. Sodium borohydride (100 mg) was added to a soln of methyl fibraurin (480 mg) in EtOH (100 ml). After being allowed to stand for 24 hr, the reaction mixture was diluted with water, then acidified with AcOH, and then extracted with $CHCl_3$. The extract was absorbed on a column of silica gel and eluted with $CHCl_3$–EtOH mixtures, ranging from 100:1 to 50:1 in composition, which afforded **4b** (120 mg) and **5** (81 mg).

Hemiacetal **4b**, m.p. 256–257° (recrystallized from EtOH). (Found: C, 64·32; H, 6·74; M^+ 390. $C_{21}H_{34}O_7$ requires: C, 64·60; H, 6·71; MW 390·42.) UV (MeOH): 211 mμ (3,900). IR(KBr): 3450, 1749 cm^{-1}. NMR ($CDCl_3$): 3·05 (1H, d, J 4·2, OH), 4·80 ppm (1H, q, J 7·5 and 4·2 c/s, 13-H).

Deoxo derivative **5**, m.p. 207° (EtOH). Found M^+, 374·170; $C_{21}H_{30}O_6$ requires: MW 374·173. UV (MeOH): 204 mμ (3,900). IR (KBr): 1765 cm^{-1}. NMR (DMSO-d_6): 3·45 ppm (2H, d, J 7·5, 13-H).

Chromic acid oxidation of the hemiacetal **4b** *to methylpalmarin.* The hemiacetal **4b** (40 mg) from 7 in pyridine (1·5 ml) was treated with chromic acid (150 mg) in pyridine (1·5 ml). The reaction mixture, after standing overnight, was diluted with MeOH (1·5 ml) and then poured into water, and extracted with $CHCl_3$. Evaporation of the extract and crystallization from MeOH afforded crystals (7 mg), m.p. 261–263°, which were identical with methylpalmarin[9] (mixed m.p., IR, TLC). Found M^+ 388·154; $C_{21}H_{24}O_{17}$ requires: MW 388·152. IR (KBr): 1766, 1730 cm^{-1}.

Tetrahydrofibraurin (2). A soln of fibraurin (200 mg) in 0·1N aqueous alkali was not hydrogenated with 10% Pd-C. Therefore, a soln in alkali which had been neutralized with dil. HCl was hydrogenated with 10% Pd-C. The reaction was stopped after absorption of 2 moles of H. Extraction of the soln with ether yielded 60 mg of **2**, which was recrystallized from MeOH, m.p. 289–293°. (Found: C, 63·58; H, 6·32. $C_{20}H_{34}O_7$ requires: C, 63·82; H, 6·43.) UV (MeOH): 231 mμ (6,500). IR (KBr): 3440, 1760, 1716, 1642 cm^{-1}. NMR (DMSO-d_6): 1·06 (6H, s, Me), 1–2·5 (8H, m, CH_2 and CH), 3·5–4 (6H, m, O—CH), 4·44 (1H, m, H-12), 5·08 (1H, broad d, J 2·8, H-1), 5·30 (1H, broad s, OH), 7·18 (1H, q, J 8 and 3, H-7).

Hexahydrofibraurin (3). Hydrogenation of **2** (46 mg) in 10 ml of dioxan and 1 ml of AcOH with 5 mg of PtO_2, removal of solvent, and recrystallization of the residue from MeOH yielded 16·8 mg of **3**, which was identical with tetrahydropalmarin[8] (mixed m.p. $[\alpha]_D$, IR, TLC).

Epoxy ketone **14**. Palmarin (130 mg) was reduced with LAH, and the oily product was oxidized with HIO_4–$NaHCO_3$ as reported;[6] the oil was chromatographed on silica gel. Elution with $CHCl_3$–EtOH mixture (100:1) afforded 47 mg of **14**, m.p. 133–134°. (Found: C, 69·00; H, 7·28. $C_{19}H_{24}O_5$ requires: C, 68·65; H, 7·28%.) IR (KBr): 3400, 1715 cm^{-1}. ORD (*c* 0·0872 in MeOH), 25°; $[\phi]_{600}$ −207, $[\phi]_{365}$ −2390, $[\phi]_{317}$ +1590, $[\phi]_{300}$ −747.

Conversion of fibraurin (**1**) *to palmarin* (**9**). Fibraurin (400 mg) was refluxed for 5 hr with 200 mg $NaBH_4$ in 60 ml of EtOH, the excess reagent was destroyed with 0·2 ml AcOH, the soln was concentrated to 20 ml and extracted with $CHCl_3$ (30 ml × 3). Evaporation of $CHCl_3$ afforded an oil (358 mg), which was further oxidized with 300 mg of anhyds CrO_3 in pyridine (5 ml). Extraction with ether (30 ml × 3) afforded 52 mg of crystals, m.p. 260°, $[\alpha]_D^{28}$ +17° (*c* 0·93 in pyridine), which were identical with palmarin (IR, TLC).

Note added in proof:

Since fibraurin (**1**) has been correlated with palmarin (see Fig. 5) no attempt was made to establish the configurations of asymmetric centers in fibraurin, with the exception of those at C_3 and C_8 which were still unknown. However, after submission of this manuscript, the absolute stereochemistry of the Colombo root principles has been reversed [K. H. Overton, N. G. Weir and A. Wylie, *J. Chem. Soc.* 1482 (1966)]. Consequently, the absolute configurations should be reversed at all centers, in the structures given in this paper, with the exception of those at C_3 and C_8 which remain as determined.

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