HIGHER ISOPRENOIDS—II

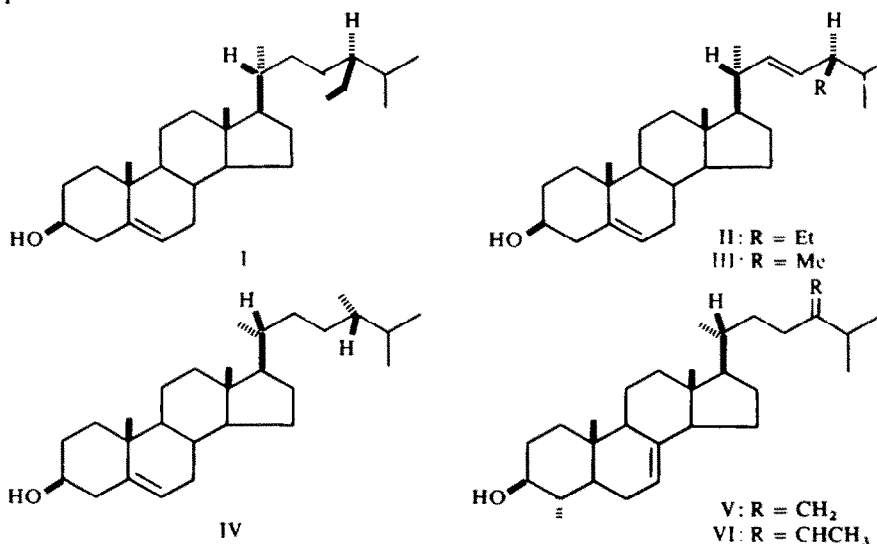## TRITERPENOIDS AND STEROIDS OF *SACCHARUM OFFICINARUM* LINN.*†

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Abstract—Besides β-sitosterol and stigmasterol, the major steroids of sugar cane, the following minor triterpenoids and steroids have been isolated and characterized from Indian sugar cane: taraxerol, β-amyrin, betulin, taraxerol methyl ether (sawamilletin), β-amyrin methyl ether (isosawamilletin), fernenol methyl ether (arundoin), isoarborinol methyl ether (cylindrin), 24-methyl- and 24-ethyl-lophenol, stigmast-5-en-3β, 7α-diol (ikshusterol), stigmast-5-en-3β, 7β-diol (epi-ikshusterol) and stigmastan-3β,5α,6β-triol.

SUGAR CANE (*Saccharum officinarum* Linn.) wax has been the subject of several investigations[1] and, a few of these[2,3] concern the characterization of sterols present in its non-saponifiable fraction. The presence of β-sitosterol (I) and stigmasterol (II) is well-established[2,3] while, the presence of brassicasterol (III) has also been claimed.[3] Recently,[4] Cuban sugar cane leaf wax has been shown to contain β-sitosterol, stigmasterol, camphesterol (IV), and minor amounts of 24-ethylidene-lophenol (VI) and 24-methylene-lophenol (V). In connection with a study aimed at utilizing sugar cane press-mud wax as a source for stigmasterol, we became interested in a detailed study of the minor isoprenoids present and we wish to report these results in the present paper.

II: R = Et
III: R = Me

IV

V: R = CH₂
VI: R = CHCH₃

Sugar cane press-mud was saponified and the sterol-rich non-saponifiable fraction (25–30% on wax) processed as detailed under Experimental to give aliphatic compounds (30–35%), sterols crystallizate (20–25%; β-sitosterol and stigmasterol) and sterols mother liquor. From the latter it has been possible to isolate taraxerol[5] and betulin[6] (Table 1) and, the triterpene methyl ethers arundoin (VII; fernenol Me ether),[13] isosawamilletin (VIII; β-amyrin Me ether),[12] sawamilletin (IX; taraxerol Me ether)[10] and cylindrin (X, isoarborinol Me ether)[13,14] (Table 2), and several new sterols (Table 1). Mother liquor from taraxerol crystallizations, yielded after methylation (CH$_2$N$_2$—HBF$_4$),[7] a material which clearly showed a spot for β-amyrin Me ether, besides that of taraxerol Me ether on TLC, indicating thereby that traces of β-amyrin accompany taraxerol fraction. Though, triterpene Me ethers, are known[9] to occur in some plants other than grasses, they appear to be characteristic[10–15] of the family *Gramineae*, of which sugar cane is a member.*
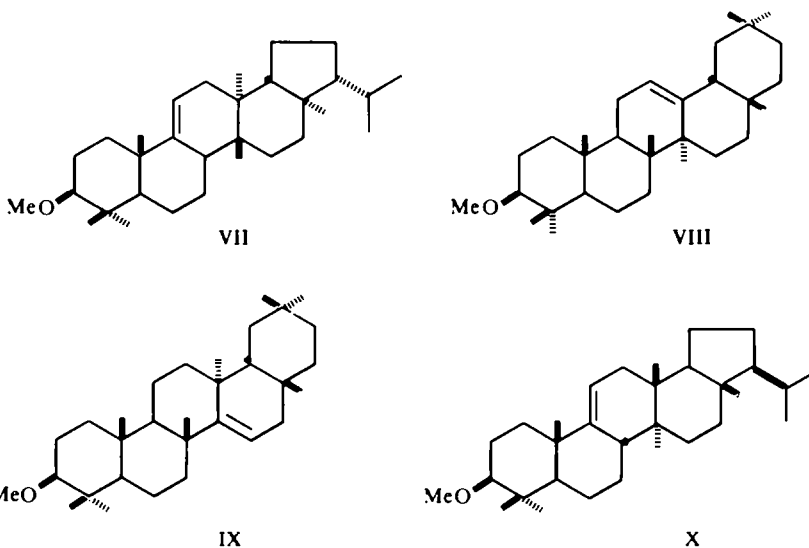
24-Methyl- and 24-ethyl-lophenol. The material corresponding to TLC spot No. 5 (Table 1) analyses for C$_{29-30}$H$_{50-52}$O and shows in its IR spectrum absorption due to OH (3433 and 1063 cm^{-1}). Its PMR spectrum displays signals assignable to two quaternary Me's (32 and 49 c/s), —C—OH (1H, diffused m centred at 182 c/s,

$$\overset{|}{H}$$

W_H = 23 c/s) and an olefinic proton (1H, diffused m centred at 310 c/s, W_H = 11 c/s). From the absorption pattern in the Me proton region of the PMR spectrum it was clear that the compound is not a triterpene and hence, must be a steroid. A comparison of the PMR spectrum with that of β-sitosterol (I) or stigmasterol (II) shows that the quaternary Me signals (32, 49 c/s) of the new compound are considerably shielded as compared to those of β-sitosterol (41, 60 c/s) or stigmasterol (43, 62 c/s). Since, due to the anisotropy of the ethylenic linkage, the position of a nuclear olefinic

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* After the present work had been completed, Bryce *et al.*[16] have reported on the isolation of arundoin and sawamilletin from Cuban sugar cane wax.

TABLE 1. ISOPRENOIDS FROM SUGAR CANE WAX

TLC* spot No.	R_s^* Solvent A	Solvent B	Mol. formula	m.p.	$[\alpha]_D^{CHCl_3}$	Remarks
1	2·76	—		160–220°		mixture of triterpene methyl ethers (Table 2)
2	2·43	--	—	-	—	could not be isolated
3	2·14	—	--	—		could not be isolated
4	1·67	—	$C_{30}H_{50}O$	273–275°	+ 1·6°	taraxerol
5	1·40	—	—	165–167°	+ 12·1°	mixture of two new sterols
6	1·00	1·00	$C_{29}H_{50}O$	137–138°	− 37·0°	β-sitosterol
6	1·00	1·00	$C_{29}H_{48}O$	167–169°	− 39·0°	Stigmasterol
7	0·64	1·00	$C_{30}H_{50}O_2$	253–254°	+ 18·6°	betulin
8	—	0·66	$C_{29}H_{50}O_2$	129–133°	− 27·0°	a new steroid diol
9	-·	0·63	$C_{29}H_{50}O_2$	211–214°	− 22·5°	a new steroid diol
10	—	0·51	—	228–229°	+ 21·6°	?
11	-·	0·36	--	210–212°	− 96·6°	?
12	--	0·21	$C_{29}H_{52}O_3$	250–252°	0°	a new steroid triol

* Silica gel-G layer (0·3 mm); solvent front: 10 cm; temp: 27°; solvent A: 1% MeOH in C_6H_6; solvent B: 40% acetone in C_6H_6.

$$R_s = \frac{\text{Movement of compound from start in mm}}{\text{Movement of β-sitosterol from start in mm}}$$

TABLE 2. TRITERPENE METHYL ETHERS FROM SACCHARUM OFFICINARUM LINN

TLC spot No.	R_{dye}^*	Mol. formula	M⁺ (m/e)	m.p.	$[\alpha]_D^{CHCl_3}$	Remarks
1	4·5	$C_{31}H_{52}O$	440	233–234°	− 4·96°	Arundoin
2	4·1	$C_{31}H_{52}O$	440	250–251°	—	Isosawamilletin
3	3·8	$C_{31}H_{52}O$	440	276–278°	+ 12·75°	Sawamilletin
4	3·5	$C_{31}H_{52}O$	440	237–243°	+ 60·0°	Cylindrin

* Silica gel-15% $AgNO_3$ (0·3 mm layer); solvent: 10% C_6H_6 in hexane; solvent front: 10 cm (3 irrigations); temp 27°.

$$R_{dye} = \frac{\text{Movement of substance from start in mm}}{\text{Movement of p-methoxy azobenzene from start in mm}}$$

linkage, the position of a nuclear olefinic linkage has a considerable effect on the C_{18} and C_{19} Me signals,[17] it is concluded that the new compound is not a Δ^5-sterol; this will also explain the different position of the CHOH signal (centred at 182 c/s) in its PMR spectrum, as compared to the positions of CHOH signals in the spectrum of β-sitosterol (centred at 210 c/s) and stigmasterol (centred at 212 c/s). In view of its quick response to Liebermann–Burchard reaction[18] and the positive SeO_2 test[19] (on the acetate), the compound is considered to be a Δ^7-sterol. This is fully supported by comparison of the obereved quaternary Me signals (32, 49 c/s) with the values (33, 48 c/s) calculated[17c] for a 3β-hydroxy-Δ^7,5α-sterol having a 17β-C_9H_{19} side-chain.

Mass spectrum of this material shows highest mass ion at $m/e = 428$ (71%)* and another peak at $m/e = 414$ (72%) indicating that the product is a mixture of $C_{30}H_{52}O$ and $C_{29}H_{50}O$; this is supported by the presence of peaks at m/e 413 (M-15; 13%) and $m/e = 399$ (M'-15; 12%). Besides these, the mass spectrum shows important peaks at m/e 287 (M$^+$-side chain $C_{10}H_{21}/C_9H_{19}$; 27%), 285 (M$^+$-$C_{10}H_{21}/C_9H_{21}$—H$_2$; 24%), 269 (M$^+$-$C_{10}H_{21}/C_9H_{19}$—H$_2$O, metastable ion at m/e 252·5, calcd 252·1; 100%), 260 (15%), 245 (M$^+$-side chain plus C_3H_6,† i.e. $C_{13}H_{27}/C_{12}H_{25}$; 27%), 227 (M$^+$-$C_{13}H_{27}/C_{12}H_{25}$—H$_2$O, metastable ion at $m/e = 210$·5, calcd 210·2; 27%), 161 (33%), 149 (15%), 147 (33%), 145 (20%), 135 (26%), 133 (27%) and 131 (18%). It is clear from these that these two homologues differ from each other in the size of the side-chain. Furthermore, the important mass peaks enumerated above are precisely the prominent peaks (besides the molecular ion peak at $m/e = 400$; 100%) in the mass spectrum of lophenol (XI) and hence it follows that the material corresponding to TLC spot No. 5 (Table 1) must be a mixture of 24-methyl- (XII) and 24-ethyllophenol

XI: R = H
XII: R = Me
XIII: R = Et
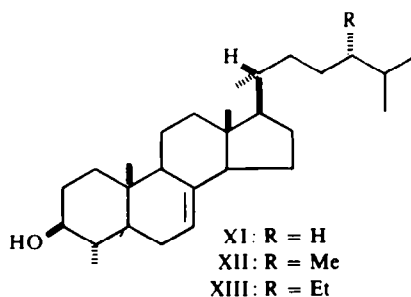
(XIII), a finding fully consistent with the spectral and other properties discussed earlier.

This material on acetylation yielded an acetate, which after a number of crystallizations gave a product showing characteristics (m.p. 142–144°, $[\alpha]_D$ +28°) close to those recently recorded[4] for the acetate of XII (m.p. 145–147°, $[\alpha]_D$ +41·3°).

Ikshusterol and epi-ikshusterol. Compounds corresponding to TLC spots No. 8 and 9 (Table 1) have close R_f values and are best separated by chromatography of the derived benzoates. These compounds have been named *ikshusterol* (TLC spot No. 8) and *epi-ikshusterol*‡ (TLC spot No. 9).

Ikshusterol, $C_{29}H_{50}O_2$, is a diol (IR: OH 3340, 1050 and 1018 cm^{-1}; dibenzoate, m.p. 168–170°, $[\alpha]_D$ −131·8°) and from its Me proton signals (PMR) which are reminiscent of β-sitosterol, the compound is expected to be a steroid. The PMR spectrum shows signals for two C$\underline{H}$OH (two overlapping multiplets centred at ～205 and 227 c/s) and one olefinic proton (broad doublet centred at 312 c/s). It gives a deep blue colour with SbCl$_3$ (in CHCl$_3$), a reaction considered to be characteristic[21,22] of 7-hydroxy-Δ^5-sterols. From all these it appeared likely that the compound may be a 7-hydroxy-β-sitosterol. That this is indeed so was established by a direct correlation with β-sitosterol, which also clarified its C$_7$-stereochemistry.

* Figures in parentheses represent % of the base peak.

† It has been emperically established[20] that the most prominent peak in the mass range 205–245, in the mass spectrum of a steroid arises from loss of side chain plus C_3H_6 (and loss of one or more molecules of H$_2$O depending on the structure of the steroid).

‡ Derived from Sanskrit *Ikshu* for sugar cane.

Oxidation of β-sitosteryl acetate with t-butyl chromate[23] furnished the desired unsaturated ketone (XIV): λ_{max} 234 mμ, ε 11,520. Its reduction with LAH yielded a mixture of diols (blue colour with $SbCl_3$), which were separated by chromatography

XIV

XV : R = H, αOH
XVI: R = H, βOH
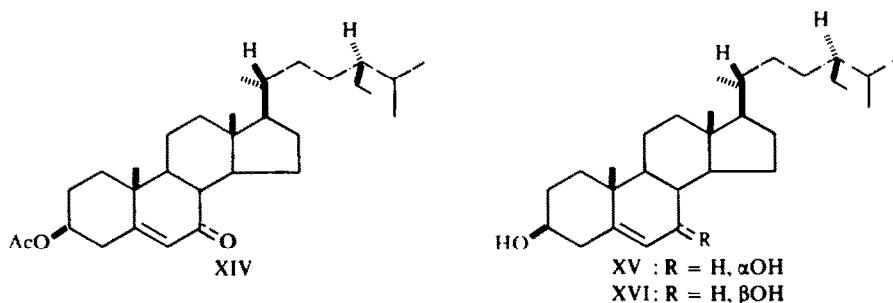
of the derived dibenzoates. The less abundant component was found to be completely identical (TLC, m.p., mixed m.p., $[\alpha]_D$, IR) with the dibenzoate of ikshusterol.

Epi-ikshusterol (deep blue colour with $SbCl_3$) furnishes a dibenzoate (m.p. 154–156°, $[\alpha]_D$ +96°) which was found to be identical (TLC, m.p., mixed m.p., $[\alpha]_D$, IR) with the major dibenzoate derived from the diols obtained by LAH reduction of XIV (*vide supra*).

In anology with the earlier experience in the cholestane[24] and the spirostan[25] series, the major product of LAH reduction of XIV is assigned the C_7-βOH configuration (XVI) and hence this must also represent epi-ikshusterol* Ikshusterol should then be XV. The conclusions are fully supported by the molecular rotational data. For the dibenzoates corresponding to the two epimers XV and XVI..

M_D (epimers) = +1453, which is close to the values reported[25] for the corresponding cholestane (+1227) and spirostan (+1234) series.

Stigmastan-3β,5α,6β-triol. This compound (TLC spot No. 12, Table 1. Liebermann–Burchard test +ve. IR: OH 3440, 1050 cm^{-1}; no C=O absorption) on acetylation (Ac_2O, pyridine) furnished an hydroxy diacetate, $C_{33}H_{56}O_5$. (IR: OH 3500, 1035 cm^{-1}; OAc 1740, 1730, 1262 and 1250 cm^{-1}), the PMR spectrum of which shows signals assignable to two quaternary Me's (s's at 53 and 66 c/s), two OCOMe (3H, s's at 118 and 122 c/s) and two —C—OAc (1H, broad s at 282 c/s; 1H, diffused m centred

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H

at 305 c/s, W_H = 18 c/s). The close similarity between this PMR spectrum and that of the 3,6-diacetate[27] derived from XVII suggested that the new triol, in fact, may be stigmastan-3β,5α,6β-triol (XVIII). This was indeed found to be so when hydroxylation (performic acid, hydrolysis) of β-sitosterol (I) yielded a product identical (m.p., $[\alpha]_D$; TLC, m.p., $[\alpha]_D$, IR of derived diacetate) with the naturally occurring triol.

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* Mitsui and Matsuda[3b] have described a steroidal diol (m.p. 206°; dibenzoate, m.p. 158–160°) of undetermined constitution, from sugar can wax and designated it α-saccharostane-diol. It is likely that this compound may in fact be epi-ikshusterol (XVI). This appears to be supported by the fact that the compound reported by these authors also gave a blue colour with $SbCl_3$.

Preparation of C_{24}-epimer of epi-ikshusterol from clionasterol has been reported.[26] The dibenzoate of this diol has properties (m.p. 159–160 , $[\alpha]_D^{CHCl_3}$ +93 4°) very close to those of epi-ikshusterol.

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XVII   XVIII
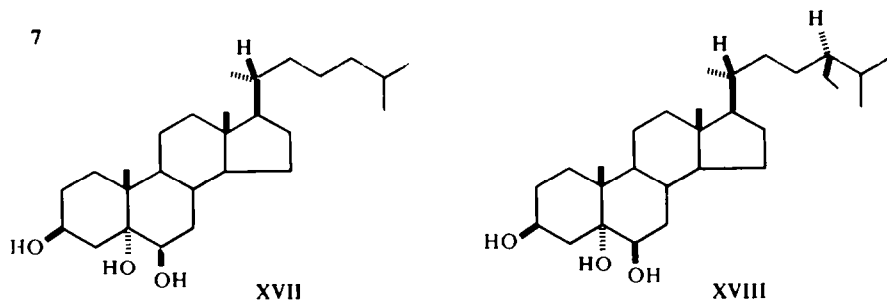
EXPERIMENTAL

All m.ps are uncorrected. Light petroleum refers to the fraction b.p. 40–60°. Optical rotations were measured in $CHCl_3$.

UV spectra were taken on a Perkin–Elmer spectrophotometer, model 350, in 95% EtOH. IR spectra were recorded as Nujol· muHs on a Perkin–Elmer Infracord model 137E. PMR spectra were taken in $CDCl_3$, unless otherwise stated, on a Varian A-60 spectrometer, using TMS as the internal standard; signals are recorded in c/s relative to TMS as zero. Mass spectra were recorded on a CEC mass spectrometer, model 21-110B, using an ionizing potential of 70 eV and a direct inlet system.

Alumina used for chromatography was washed neutral,[28] activated at 440–460° (6 hr) and standardized according to Brockmann.[29] Silica gel for column chromatography was of 100–200 mesh and was activated at 130–140° (6 hr) and then standardized.[30] $AgNO_3$-impregnated silica gel was made by the method of Gupta and Dev[8] and activated at 100–110° (4 hr). TLC was carried out on silica gel or silica gel-$AgNO_3$ (7·5% $AgNO_3$) layers (0·3 mm) containing 15% gypsum; the layers were activated at 100–110° (45 min) and then stored in a desiccator. Conc H_2SO_4-spray, followed by heating (120°, 5 min) was used for visualization of TLC spots.

Liebermann–Burchard test was carried out by adding conc H_2SO_4 (2 drops) to a soln of the sample (2 mg) in $CHCl_3$-Ac_2O (1 ml each); β-sitosterol was used for comparison purposes.

Saponification and preliminary separation of sterols. The press mud wax* (500 g) in C_6H_6–EtOH (750 ml each) was refluxed (24 hr) with KOHaq (18·5 N, 100 ml) with stirring. The solvent was flashed off under reduced pressure (water bath) and the residue, well-powdered and exhaustively percolated with ethylene dichloride at room temp to get a sterol-rich extract (140 g). This material (100 g) was digested with acetone (600 ml) at reflux for 1·5 hr, cooled to room temp and filtered to remove the insoluble material. The insoluble material was treated with acetone as before, twice again; the final "acetone-insoluble" material was essentially free of sterols (Liebermann–Burchard test). The three acetone extracts were combined and stripped off the solvent to give a sterol concentrate (64 g) as a semi-solid dark mass. Crystallization of this material (60 g) from light petroleum (600 ml) gave in two crops a sterol crystallizate (21·1 g, m.p. 127–134°; mixture of β-sitosterol and stigmasterol) and, a thick oil (38·2 g) after removing solvent from the mother liquor.

The above oil (50·0 g) from the mother liquors was taken up in light petroleum (100 ml) and chromatographed on silica gel (grade I-IIA; 1200 g, 35 cm × 8 cm) with TLC monitoring (Table 1):

Fraction 1	light petroleum	6 × 600 ml	8·4 g, essentially aliphatic hydrocarbons; rejected
Fraction 2	C_6H_6	10 × 600 ml	6·0 g, Compound 1*
Fraction 3	2% MeOH in C_6H_6	25 × 600 ml	31·4 g, compounds 4, 5, 6 and 7
Fraction 4	10% MeOH in C_6H_6	4 × 600 ml	7·1 g, Compounds 8, 9, 10 and 11
Fraction 5	20% MeOH in C_6H_6	3 × 600 ml	0·73 g, compound 12
Fraction 6	MeOH	8 × 600 ml	1·9 g, complex mixture, not investigated further

* These numbers refer to the TLC spot Nos., Table 1.

Triterpene methyl ethers. Fraction 1 (6·0 g), a brownish red semi-solid, on trituration with cold acetone

* Supplied by Ravalgaon Sugar Farm, Ravalgaon. The wax was obtained by extraction of press-mud by petrol, fraction b.p. 120–150° and, was greenish brown in colour with m.p. 75–83°.

(25 ml) yielded a solid* (0·698 g, m.p. 160–220°), consisting essentially of triterpene methyl ethers. This material (1·4 g) was chromatographed on AgNO$_3$–silica gel (10% AgNO$_3$; 61 cm × 1·6 cm) using a single solvent system (10% C$_6$H$_6$ in pet. ether) and TLC monitoring (Table 2):

Fraction 1a	10 ml × 48	—
Fraction 1b	5 ml × 14	76 mg, m.p. 220–225° compound 1*
Fraction 1c	5 ml × 2	44 mg, compounds 1 and 2
Fraction 1d	5 ml × 9	27 mg, m.p. 220–230°, compound 2
Fraction 1e	5 ml × 3	18 mg, compounds 2 and 3
Fraction 1f	5 ml × 25	363 mg, m.p. 258–266°, compound 3
Fraction 1g	5 ml × 134	844 mg, compounds 3 and 4

  * These numbers refer to the TLC spot numbers, Table 2.

Arundoin (VII). Fraction 1b on crystallization from C$_6$H$_6$ yielded a product m.p. 233–234° (Table 2). PMR: 3H Me signals at 44·7, 46·5, 50·0, 50·5, 50·5, 57·5, 57·5 and 63·5; —CHOMe (1H, m centred at 160 c/s, W_H = 17 c/s); OMe (3H, s at 202 c/s); olefinic proton (1H, diffused d centred at 321 c/s). (Lit.[13] m.p. 235–237°, 242–243°, [α]$_D$ −5·3°).

Isosawamilletin (VIII). Recrystallization of fraction 1d from CHCl$_3$–MeOH yielded 7 mg of pure VIII, m.p. 250–251° (Table 2), which was compared (TLC, mixed m.p.) with an authentic sample.

An authentic sample of VIII was obtained as follows. To β-amyrin (35 mg, m.p. 192–194°) in CH$_2$Cl$_2$ (5 ml) containing two drops of HBF$_4$ (d = 1·36, 2 drops) was added at −5° to 0° a chilled soln of CH$_2$N$_2$ in CH$_2$Cl$_2$ (from 560 mg of nitrosomethyl-urea, 1 g KOH, 1 ml H$_2$O and 6·5 ml CH$_2$Cl$_2$). After 80 min at the same temp the reaction mixture was worked up in the usual manner and the product purified by chromatography to give 12 mg of β-amyrin methyl ether, m.p. 248–250° (CHCl$_3$–MeOH) (Lit.[16], m.p. 247–248°).

Sawamilletin (IX). Recrystallization of fraction 1f from C$_6$H$_6$ furnished a product m.p. 276–278° (Table 2). This compound can also be obtained directly from the original Me ether mixture by its repeated crystallization from hot C$_6$H$_6$; PMR: quaternary Me signals at 46, 49, 55, 55, 55, 57, 57 and 65 c/s; —CHOMe (1H, diffused m centred at 158 c/s), OMe (3H, s at 200 c/s); olefinic proton (1H, diffused m centred at 332 c/s).

An authentic sample of IX was prepared by methylation of taraxerol (40 mg) by the above procedure: m.p. 275–277° (C$_6$H$_6$) (Lit.[10] m.p. 278°).

Cylindrin (X). Fraction 1g (844 mg) was recrystallized from light petroleum (20 ml) and two further crops obtained by concentrating the mother liquor each time. The final mother liquor was free from sawamilletin (TLC) and on solvent removal gave crude cylindrin (164 mg, m.p. 237–243°). This material was identified as cylindrin by PMR and mass spectrometry. However, the m.p. could not be improved by crystallizations from light petroleum, C$_6$H$_6$ or CHCl$_3$–MeOH (Lit.[14] m.p. 269–270°, [α]$_D$ +60°), though mixed m.p. with an authentic sample (m.p. 258–260°) was raised to 258–260°. PMR: Me signals at 47·5–48·5 (~15H), 58·6 (6H) and 63 c/s (3H); CHOMe (1H, m centred at 161, W_H = 20 c/s); OMe (3H, s at 203 c/s); olefinic proton (1H, m centred at 315 c/s).

Isolation of β-sitosterol, stigmasterol, taraxerol, betulin and, 24-methyl and 24-ethyl-lophenol mixture. Fraction 3 of the initial chromatography (31·3 g) was crystallized from acetone (100 ml) to give a mixture (13·3 g, m.p. 133–136°) shown by chemical separation[31] to consist of β-sitosterol (~92%; m.p. 137–138°) and stigmasterol (~8%; m.p. 167–169°).

The mother liquor from the above was stripped off the solvent and the residue (thick oil, 18·0 g) chromatographed over silica gel (grade I–IIA; 550 g, 22 cm × 6·5 cm) with TLC monitoring (Table 1):

Fraction 3a	C$_6$H$_6$	200 ml × 10	376 mg oil
Fraction 3b	3% EtOH in C$_6$H$_6$	200 ml × 7	5·1 g, red viscous material containing compound 4*
Fraction 3c	4% EtOH in C$_6$H$_6$	200 ml × 6	3·5 g, wax, containing compounds 4, 5 and 6
Fraction 3d	6% EtOAc in C$_6$H$_6$	200 ml × 10	3·17 g, m.p. 131–135°, mixture of β-sitosterol and stigmasterol (compound 6)
Fraction 3e	7% EtOAc in C$_6$H$_6$	200 ml × 5	793 mg, wax containing compound 7
Fraction 3f	EtOAc	200 ml × 10	4·5 g, viscous mass, no crystalline product could be isolated

  * These numbers refer to the TLC spot numbers, Table 1.

  * It must be mentioned that TLC single spot materials corresponding to various TLC spots (Table 1) gave only poor yields of crystalline materials and considerable quantities of viscous oils/gums showing practically no fine structure in the IR spectra.

Taraxerol. Fraction 3b (5·1 g) in light petroleum (20 ml) slowly deposited a crystalline solid (200 mg, m.p. 273–275°; Table 1): Liebermann–Burchard test, persistent purple colour; IR: OH 3475 and 1040 cm^{-1}; —C=CH 820 cm^{-1}; PMR: Me signals at 49–50 c/s (6H), 55–56 (12H), 59 (3H) and 66 (3H); CHOH

(1H, broad m centred at 192 c/s); —C=CH— (1H, broad m centred at 333 c/s). Mass $(m/e =)$: 426 (M$^+$, 40%), 411 (10%), 302 (55%), 287 (20%), 218 (24%), 204 (100%). (Found: C, 84·14; H, 11·87. C$_{30}$H$_{50}$O requires: C, 84·50; H, 11·53%). The compound was acetylated (Ac$_2$O, pyridine) to give a product, m.p. 295–298°, $[\alpha]_D$ +2·0° (c 5·2%); mixed m.p. with taraxeryl acetate (m.p. 295–297°) remained undepressed. (Lit.[5]: taraxerol, m.p. 282–285°, $[\alpha]_D$ ±0°; acetate, m.p. 303–305°, $[\alpha]_D$ +11).

Betulin. Fraction 3e (790 mg) in light petroleum (13 ml) slowly furnished a solid (95 mg, m.p. 165–185°), which on repeated crystallization from EtOH yielded colourless crystals (40 mg), m.p. 253–254° (Table 1): Liebermann–Burchard test, pink colour (fast); IR: OH 3400, 1040–1010 cm^{-1}; —C=CH$_2$ 1640 and

885 cm^{-1}; PMR: quaternary Me signals at 44, 49, 58·5, 58·5 and 60·5 c/s; vinylic Me (3H, broad s at 100 c/s), —CH$_2$OH, —CHOH (3H, overlapping m's located between 178–235 c/s), —C=CH$_2$ (2H, m

located between 270–282 c/s). (Found: C, 81·47; H, 11·42. C$_{30}$H$_{50}$O$_2$ requires: C, 81·44; H, 11·31%). Acetate (Ac$_2$O-pyridine), m.p. 217–220° (EtOH), mixed m.p. with authentic butelin diacetate (m.p. 216–220°) was undepressed (Lit.:[32] butelin, m.p. 261°, $[\alpha]_D$ +20°; acetate, m.p. 223°).

24-Methyl- and 24-ethyl lophenol (XII, XIII). Rechromatography (silica gel gràde I–IIB; 90 g) of fraction 3c, (3·5 g), using 1%, 2% and 5% EtOAc in C$_6$H$_6$ as eluting solvents, gave a fraction (1·0 g) eluted with 2% EtOAc in C$_6$H$_6$ (50 ml × 5), essentially free of TLC components 4 and 6 (Table 1). This was taken up in acetone (10 ml) and diluted with CH$_3$CN (6 ml) and the crystalline solid (350 mg, m.p. 120–145°) collected after several hours. Six recrystallizations from acetone-acetonitrile yielded crystals (60 mg), m.p. 165–167° (Table 1): Liebermann–Burchard test, deep pink → deep violet → deep blue → deep green (fast). (Found: C, 83·96; H, 12·17. C$_{29}$H$_{50}$O requires: 83·99; H, 12·15. C$_{30}$H$_{52}$O requires: C, 84·04; H, 12·23%).

Ikshusterol (XV), *epi-ikshusterol* (XVI) *and compounds* 10, 11 (*Table* 1). Fraction 4 (7·0 g) of initial chromatography was rechromatographed over silica gel (grade I–IIa; 210 g, 27 cm × 4 cm) with TLC monitoring (Table 1):

Fraction 4a	10% EtOAc in C$_6$H$_6$	100 ml × 15	0·7 g, rejected
	30% EtOAc in C$_6$H$_6$	100 ml × 5	
Fraction 4b	50% EtOAc in C$_6$H$_6$	100 ml × 5	1·3 g, viscous mass containing traces of compound 8*
Fraction 4c	50% EtOAc in C$_6$H$_6$	100 ml × 3	1·0 g, mixture of compounds 8 and 9
Fraction 4d	50% EtOAc in C$_6$H$_6$	100 ml × 3	1·0 g, compound 10
Fraction 4e	50% EtOAc in C$_6$H$_6$	100 ml × 3	0·79 g, compound 11
Fraction 4f	MeOH	100 ml × 7	1·96 g, no crystalline product could be obtained

* These numbers refer to TLC spot Nos., Table 1.

Compound 10 (*Table* 1). Fraction 4d (1·0 g) in acetone was diluted with CH$_3$CN, when slowly a solid (50 mg) separated, which was thrice crystallized from EtOH to give pure compound 10 (13 mg), m.p. 228–229°. (Found: C, 80·24; H, 12·79%). Liebermann–Burchard test: pink → violet → green.

Compound 11 (*Table* 1). Fraction 4e (790 mg) in acetone (4 ml) was diluted with CH$_3$CN (2 ml) and chilled in an ice-bath. The solid (130 mg, m.p. 195–202°) was collected and recrystallized from EtOH to give shining crystals (60 mg), m.p. 210–212°. (Found: C, 78·50; H, 11·85%). Liebermann–Burchard test: dirty brown.

Ikshusterol (XV) *and epi-ikshusterol* (XVI). Fraction 4c (1·3 g) was benzoylated (benzoyl chloride 3 ml, pyridine 7 ml; 15 hr, room temp) and the crude product (gum, 2 g) chromatographed on Al$_2$O$_3$ (grade II; 100 g, 22 cm × 2 cm) with TLC monitoring (solvent: 30% light petroleum in C$_6$H$_6$). 20% benzene in light petroleum (20 ml × 13) eluted compound 8 (ikshusterol) benzoate (400 mg) which was once crystallized from EtOHaq and then thrice from EtOH to give pure ikshusterol benzoate (23 mg, m.p. 168–170°). (Found: C, 80·54; H, 9·07. C$_{43}$H$_{58}$O$_4$ requires: C, 80·83; H, 9·15%). Next, elution with 20% benzene in light petroleum (20 ml × 5) and 50% benzene in light petroleum (20 ml × 3) eluted a mixture (260 mg) of compound 8 and 9 dibenzoates. Finally, 50% benzene in light petroleum (20 ml × 2) and benzene

(20 ml × 7) eluted 560 mg of compound 9 (epi-ikshusterol) dibenzoate, which after recrystallization from EtOH gave a pure product (51 mg), m.p. 154–156°. (Found: C, 80·69; H, 9·06. $C_{43}H_{58}O_4$ requires: C, 80·83; H, 9·15%).

In another experiment fraction 4c (1·0 g) was dissolved in acetone (10 ml) and diluted with water, chilled and the waxy product (170 mg) collected. This was filtered through Al_2O_3 (grade II) using 2% MeOH in C_6H_6 as solvent. This product (83 mg, m.p. 139–144°) was separated by chromatography on silica gel to furnish ikshusterol (compound 8, Table 1; 23 mg. Liebermann–Burchard test: deep pink → deep violet → deep green. (Found: C, 80·67; H, 11·43. $C_{29}H_{50}O_2$ requires: C, 80·87; H, 11·70%) and epi-ikshusterol (compound 9, Table 1; 29 mg. Liebermann–Burchard test: same as ikshusterol. (Found: C, 80·63; H, 11·45. $C_{29}H_{50}O_2$ requires: C, 80·87; H, 11·70%). It was ascertained (TLC) that these sterols on benzoylation furnish the dibenzoates described above.

3β-*Acetoxy-5-stigmasten-7-one* (XIV). To a refluxing soln of β-sitosteryl acetate (3·0 g) in dry CCl_4 (10 ml) was introduced (45 min) the oxidizing mixture (t-butyl chromate[23b] 30 ml, AcOH 10 ml and Ac_2O 5 ml) with mechanical stirring, under anhyd conditions. After stirring and refluxing for 10 hr, the reaction mixture was cooled (5–10°) and treated cautiously (45 min) with vigorous stirring with oxalic acid aq (9 g in 90 ml H_2O). The mixture was stirred at room temp for another 2 hr and worked up as usual.[23b] The crude product (3·2 g) in C_6H_6 (4 ml) and light petroleum (16 ml) was chromatographed over grade IIB silica gel (100 g, 29 cm × 2·9 cm) using solvent in the sequence: light petroleum (100 ml × 6; 13 mg), C_6H_6 (100 ml × 6; 333 mg), 1% EtOAc in C_6H_6 (100 ml × 9; 1·788 g, m.p. 170–174°), 5% EtOAc in C_6H_6 (100 ml × 8; 333 mg, gum), 15% EtOAc in C_6H_6 (100 ml × 4; 169 mg, gum) and EtOAc (100 ml × 4; 524 mg, gum). The product eluted with 1% EtOAc in C_6H_6, was recrystallized from acetone–MeOH to give pure XIV (1·55 g), m.p. 177–179°, $[\alpha]_D$ −97·8°. (Found: C, 79·11; H, 10·51. $C_{31}H_{50}O_3$ requires: C, 79·10; H, 10·71%); IR: OAc 1736, 1266 and 1245 cm^{-1}; —C=C—C=O 1693 and 1645 cm^{-1}.

3β,7α-*Benzoxy-5-stigmastene* (*ikshusterol dibenzoate*) and 3β,7β-*benzoxy-5-stigmastene* (*epi-ikshusterol dibenzoate*). The above product (400 mg) in dry ether (15 ml) was reduced with LAH (44 mg) in 10 ml dry ether) at room temp for 3 hr and the reaction mixture worked up with Rochelle salt soln in the usual manner to give a mixture (TLC) of diols (m.p. 143–165°). This material (400 mg) was benzoylated and the product separated by chromatography over Al_2O_3 as already described to give 111 mg of 3β,7α-benzoxy-5-stigmastene (recrystallized from acetone–MeOH, m.p. 167–169°, $[\alpha]_D$ −132°) and 311 mg of 3β,7β-benzoxy-5-stigmastene (recrystallized from acetone–MeOH, m.p. 154–156°, $[\alpha]_D$ +93·0°).

Isolation of stigmastan-3β,5α,6β-triol (XVIII). Fraction 5 (720 mg) of initial chromatography was triturated with acetone (5 ml) CH_3CN (4 ml) and the precipitate (125 mg, m.p. 237–244°) recrystallized from EtOH to give pure compound 12 (Table 1), m.p. 250–252°, $[\alpha]_D$ ∼0°, yield 80 mg; Liebermann–Burchard test, pink → blue → green (last). (Found C, 77·50 H, 11·71. $C_{29}H_{52}O_3$ requires C, 77·62 H, 11·68°/$_{o}$). *Acetate* (Ac_2O, pyridine), purified by PLC was obtained as a foam (m.p. 76–78°). $[\alpha]_D$ −36°. (Found C, 74·39; H, 10·67. $C_{33}H_{56}O_5$ requires: C, 74·39; H, 10·59%).

Hydroxylation of β-sitosterol. β-Sitosterol (200 mg) was hydroxylated with performic acid, following the conditions reported[33] for the hydroxylation of cholesterol. The crude triol (185 mg, m.p. 236–242°), so obtained, was recrystallized from EtOH to give pure XVIII, m.p. 247–249°, $[\alpha]_D$ ∼0°; *acetate*, foam (m.p. 78–80°), $[\alpha]_D$ −39·3°.

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