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CHEMISTRY OF *AYURVEDIC* CRUDE DRUGS*—I

GUGGULU (RESIN FROM *COMMIPHORA MUKUL*)—1: STEROIDAL CONSTITUENTS†‡

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Abstract—Guggulu, the gum-resin exudate from the tree *Commiphora mukul* is a complex mixture of steroids, diterpenoids, aliphatic esters, carbohydrates and a variety of inorganic ions, besides minor amounts of sesamin and other unidentified constituents. The present communication discusses in some detail the steroidal constituents, which include, cholesterol, 4,17(20)-(*trans*)-pregnadiene-3,16-dione (I), 4,17(20)-(*cis*)-pregnadiene-3,16-dione (II) and three new sterols—guggulsterol-I, guggulsterol-II and guggulsterol-III which are shown to be VIII, XV and XVI respectively.

GUGGULU (SANSKRIT) IS THE gum-resin exudate from the tree *Commiphora mukul* (Hook, ex Stocks) Engl. (Syn. *Balsamodendron mukul* Hook, ex Stocks) and is an article of commerce in India.[1] The classical Ayurvedic literature claims *guggulu* to be efficacious in the treatment of rheumatoid arthritis, obesity and allied disorders, besides indicating for it several other therapeutic uses.[2] Recent pharmacological studies on the crude drug as well as (in some cases) on some of its fractions and pure constituents, have revealed significant anti-inflammatory, anti-rheumatic[3,4] and hypocholestremic/hypolipaemic[5–8] activity, thus providing at least some support to the ancient claims.

The gum-resin is known to furnish an essential oil (~0·4%) consisting chiefly of myrcene and "dimyrcene" (camphorene).[9] It has also been separated by alcohol extraction into a soluble resin (~50%) and an insoluble carbohydrate gum;[10] detailed structural investigations on the carbohydrate gum have been reported.[10–11] It has also been noted[12] that the resin from *Commiphora mukul* is completely devoid of triterpenoids in contrast to the resin from related species *Commiphora glandulosa* Schinz which contains a number of triterpene acids.[13]

For the present investigation, the *guggulu* gum-resin was fractionated by successive solvent triturations into a pet. ether fraction (9–11%), an EtOAc fraction (32–35%) and an EtOAc insoluble residue (54–59%). The EtOAc insoluble residue was found to be free from any glycosides, and is essentially a carbohydrate polymer with a high

* Ayurveda is the ancient Indian system of treating body disorders and infections and is still freely practised in India. Though, almost always, a number of crude drugs go into formulating a specific remedy, quite often one crude drug forms the basis. Many of these drugs have been the subject of scientific investigations, but usually in a rather disjointed manner. In the present series, it is proposed to discuss the chemistry of some of those single drugs which have received support from recent pharmacological/clinical investigations.

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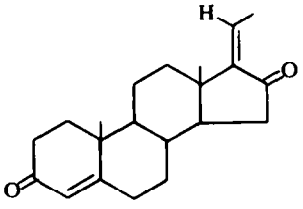
Some local names: Hindi, *guggul*; Marathi, *guggule*.

(~15%) ash content and, in view of the earlier work of Bose and Gupta[10,11] and its toxic character,[14] was not investigated further.

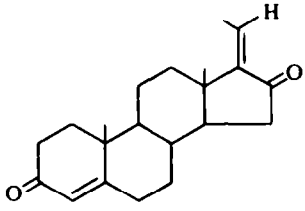
Petrol ether fraction

Systematic chromatography of pet. ether soluble fraction gave, besides some intractable mixtures, a diterpene hydrocarbon ($C_{20}H_{32}$, liq.; 8%*), a diterpene alcohol ($C_{20}H_{34}O$, m.p. 37–38°; 27%), (+)-sesamin[15] (1·2%), cholesterol† (~2%) and two other isomeric $C_{21}H_{28}O_2$ steroids of m.p. 192–193° (5%) and 168–170° (1·2%). The two diterpenes appear to be new and their structure elucidation will be reported in a subsequent communication.

The $C_{21}H_{28}O_2$ (M^+, *m/e* 312) steroid of m.p. 192–193°, from its spectral characteristics (UV, IR, PMR) has been formulated as 4,17(20)-(*trans*)-pregna-3,16-dione (I), a compound which has recently been synthetically prepared.[18] Though a direct comparison has not been possible, comparison of the physical characteristics (Experimental) and spectral data with that reported in the literature[18] leaves no doubt as to their identity. This was further confirmed by its conversion (Li/liq. NH_3 and then Sarett oxidation) to the known[19] 5α-pregnan-3,16-dione.



I



II

The isomeric $C_{21}H_{28}O_2$ (M^+, *m/e* 312) steroid of m.p. 168–170° was likewise shown to be the *cis*-isomer (II).[18]

Though the natural occurrence of C_{21} steroids is well-known,[20] compounds I and II are being reported occuring in nature for the first time and in accordance with the usual practice are being assigned trivial names, *Z*- and *E-guggulsterone* respectively.*

Ethyl acetate fraction

This material has a high ester number and is very complex in nature and failed to give any useful results on chromatography (SiO_2 gel). Hence, it was saponified and the neutral product (~55%) systematically chromatographed to furnish, besides additional quantities of *Z*-guggulsterone (0·6%†) and *E*-guggulsterone (0·5%), three new sterols (~1·2%) and long-chain aliphatic triols (15–20%). The chemistry of the

* Yields computed from chromatography data and are very approximate; the percentage is based on the pet. ether fraction on w/w basis.

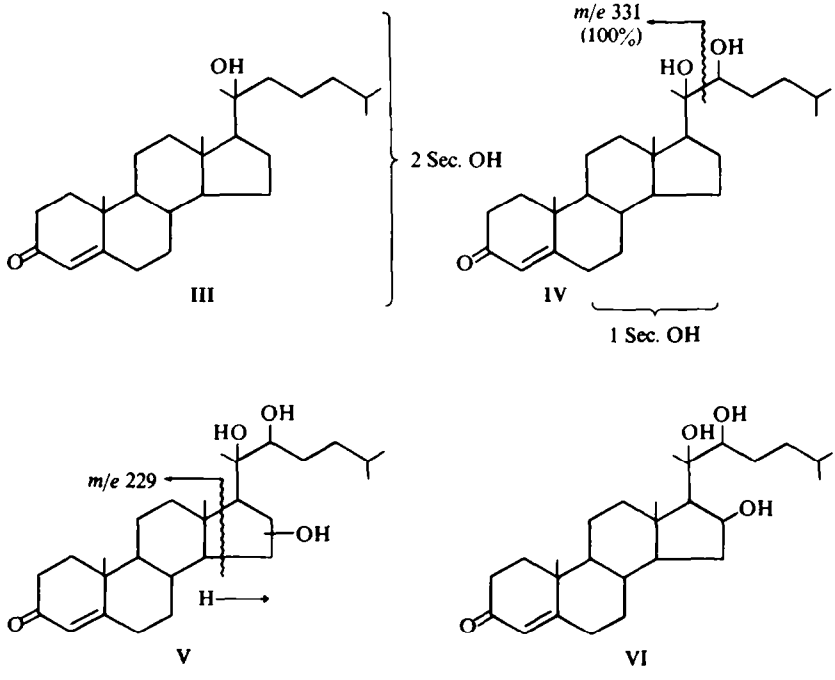
† The occurrence of cholesterol in plant tissues is quite rare and has been noted relatively recently.[16] *Commiphora abyssinica* is the only *Commiphora* species known so far to contain cholesterol.[17]

* The prefixes *Z* and *E* refer to the stereochemistry of the 17(20)-olefinic linkage, according to a recent general proposal.[21]

† These yields are per cent of the total EtOAc fraction (w/w basis).

aliphatic triols as well as the nature of the acids (obtained on saponification of the EtOAc fraction) will be discussed in a later publication. The three new sterols have been designated *guggulsterol*-I, *guggulsterol*-II and *guggulsterol*-III and their structure elucidation will now be discussed.

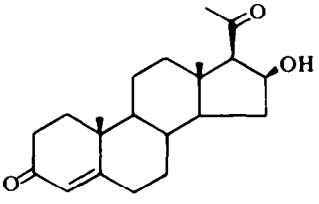
Guggulsterol-I. This compound (m.p. 225–228°) analyses for $C_{27}H_{44}O_4$ (M^+, *m/e* 432) and shows the following structural features: three Me—C— (PMR in $CDCl_3$: 9H, s, 1·21 ppm), Me_2CH— (PMR: 6H, *d*, 0·92 ppm, J = 6 Hz), two CHOH (PMR: two 1H, ill-defined multiplets centred at 3·86 and 4·45 ppm. IR (Nujol): 3300, 1080 cm^{-1}) and —CO—CH=C— (λ_{max}^{EtOH} 241 nm; ε, 16,650. IR: 1620, 1680 cm^{-1}. PMR: 1H, s, 5·75 ppm). From the mol. formula and functionality revealed above, it is obvious that guggulsterol-I should be tetracyclic and from the nature of the Me signals a steroid nucleus appeared most likely, in which case the fourth oxygen function must be a tertiary OH. From these considerations, part-structure III appeared quite reasonable, the tertiary OH being placed at C_{20} in view of the presence of three Me—C— groups in guggulsterol-I.



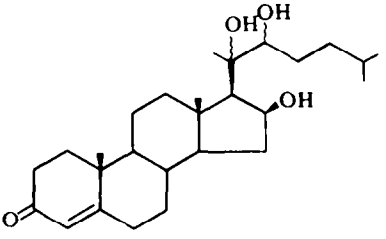
The electron impact induced fragmentation of guggulsterol-I, not only supports formulation III, but also helps in the location of the two secondary OH's. The spectrum shows the base peak at *m/e* 331, arising from cleavage of the side-chain as shown in IV and, this requires that one of the secondary OH's must be placed at C-22

and the second sec OH must be located on one of the rings. That the preferred mode of fragmentation of an α-glycol should involve the bond joining the two OH's, is theoretically expected and has experimental validity.[22] The next major ion, *m/e* 313 (66%) apparently arises from a similar cleavage of (M-18) ion (414–101). The occurrence of an ion at *m/e* 229 (9%) suggests that the ring sec OH should be at C-15/C-16, as the *m/e* 229 ion can arise by the well-established and fairly general steroid fission[23] depicted in V. These considerations lead us to two possible gross structures (VI and the alternative with ring OH at C-15) for guggulsterol-I. Of these VI is preferred because of the pattern of oxidation found in *Z*- and *E*-guggulsterone, which co-occur in the gum-resin. Decisive evidence in support of structure VI, which also helps in the elucidation of the stereochemistry at C-16 and C-17, was obtained as follows.

Guggulsterol-I on interaction with $NaIO_4$ yielded essentially two products, which after chromatographic separation were identified as iso-caproic aldehyde (by comparison of the m.p., IR and PMR spectra of its 2,4-dinitrophenylhydrazone with those of an authentic sample) and 16β-hydroxyprogesterone (VII). Though an



VII



VIII

authentic sample of VII could not be obtained, identity of the cleavage product from guggulsterol-I with 16β-hydroxyprogesterone (VII) was established by comparison of its m.p., $[\alpha]_D$, UV, IR and mass spectra with those reported in the literature.[24] This degradation clearly defines guggulsterol-I as VIII, in which the C-20, C-22 stereochemistry is yet to be elucidated.

Guggulsterol-II. This compound (m.p. 231–233°) analyses for $C_{27}H_{46}O_3$ ($M—H_2O$ ion, *m/e* 400) and shows in its IR spectrum (Nujol) OH absorption (3350, 1055 and 1045 cm^{-1}), but no C=O absorption. On exposure to Ac_2O in pyridine at room temperature (12 hr), it furnishes a diacetate (m.p. 179–181°), $C_{31}H_{50}O_5$ (M—AcOH ion, *m/e* 442), showing in its IR (Nujol) spectrum OH absorption (3590 and 1045 cm^{-1}) besides the expected AcO absorptions (1745, 1742, 1255 and 1235 cm^{-1}). Thus, guggulsterol-II is a triol having possibly one tert-OH.

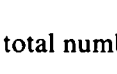
The compound is sparingly soluble in $CHCl_3$, hence its PMR spectrum was investigated in pyridine showing an $\underline{Me}_2CH—$ (6H, d, 0·86 ppm, J = 6 Hz) and three

$$\underline{Me}—\overset{|}{\underset{|}{C}}—$$

(3H, s, 1·07 ppm; 6H, s, 1·44 ppm). The PMR spectrum (CCl_4) of the diacetate displays besides $\underline{Me}_2CH—$ (6H, d, 0·93 ppm, J = 6 Hz), three $\underline{Me}—\overset{|}{\underset{|}{C}}—$

(3H singlets at 1·1, 1·8 and 1·25 ppm) and two $C\underline{H}_3COO$ (3H singlets at 1·96 and 2·04 ppm) absorptions, signals for two —C<u>H</u>OAc (two ill-defined 1H multiplets centred at 4·43 and 5·13 ppm) and one olefinic H (an ill-defined triplet at 5·31 ppm).

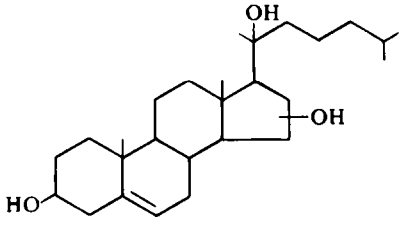
All these features are consistent with guggulsterol-II being a C_{27} steroid having two secondary and one tertiary OH, the latter being placed at C-20 to account for the



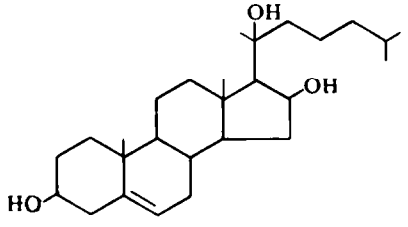
total number of <u>Me</u>—C— groups.

Oxidation of guggulsterol-II with CrO_3 in a two phase system[25] proved complex and only one compound could be obtained TLC pure, though as a gum and in a poor yield. This product shows: λ_{max}^{EtOH} 248 nm (ε, 15,470); IR (CCl_4), OH (3490 cm^{-1}), C=O (1730 and 1690 cm^{-1}). These results are interpreted in favour of structure IX for guggulsterol-II. Mass spectral fragmentation of the triol as well as that of the diacetate are fully consistent with this and the most important fragmentations are depicted in XI–XIII; the *m/e* 254 is an important ion in the mass spectrum of the acetate and appears to arise from the M-2 AcOH ion by the fragmentation shown in XIII.

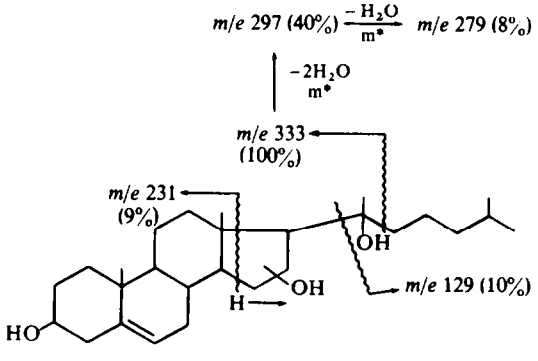
In view of the structure of guggulsterol-I, the D-ring sec OH is placed at C-16 in preference to the C-15. This speculation (X) has received full support from a partial synthesis of guggulsterol-II, which is described below:



IX

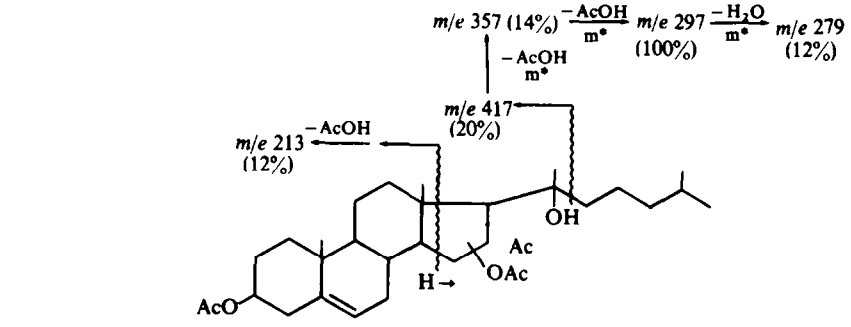


X

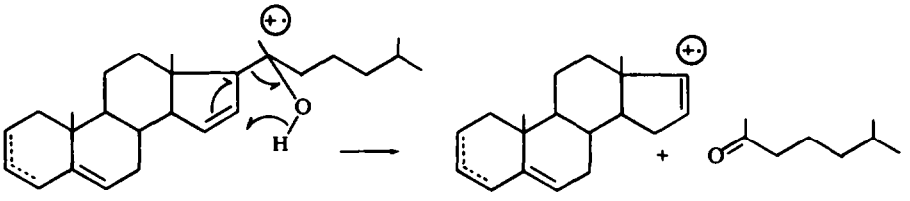


XI

The keto ester XIV available from pseudo-diosgenin diacetate[26] by CrO_3 oxidation[27] was reacted with isohexyl magnesium bromide (10 mole equiv.) in refluxing benzene to furnish a product which after saponification and chromatography



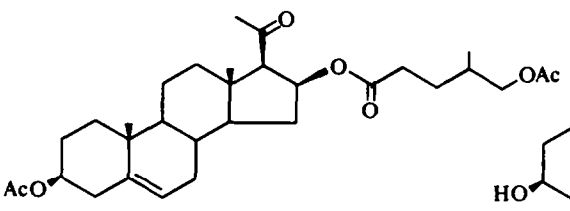
XII



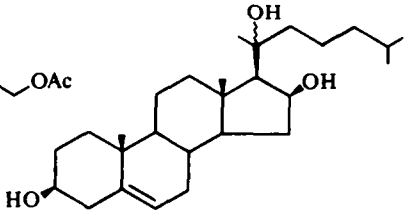
m/e 254
(33%)

XIII

afforded XV, indistinguishable (m.p., m.m.p., [α], and m.p., m.m.p., IR, PMR spectra of the derived diacetate) from guggulsterol-II. This partial synthesis from diosgenin not only confirms the formulation X for this sterol, but also clarifies the stereochemistry at various centres (except C-20) as depicted in XV.



XIV



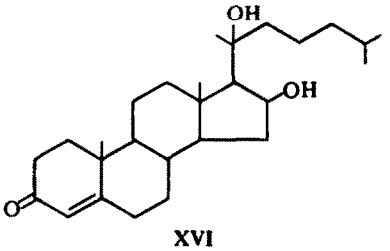
XV

Guggulsterol-III analyses for $C_{27}H_{44}O_3$ ($M-H_2O$ ion, *m/e* 398) and displays the following structural features: OH (IR in $CHCl_3$: 3400 cm^{-1}), —CO—CH=C— (|) (λ_{max}^{EtOH} 241 nm; ε, 17,140. IR: 1667, 1620 cm^{-1}. PMR in $CDCl_3$: 1H, s, 5·73 ppm), CHOH (PMR: 1H, m, 4·63 ppm), Me_2CH— (PMR: 6H, d, 0·88 ppm, J = 6 Hz) and (|) three Me—C— (PMR: 6H, s, 1·21 ppm; 3H, s, 1·28 ppm). In view of the structures (|) established for guggulsterol-I (VIII) and guggulsterol-II (XV) and the structural information outlined above, guggulsterol-III is considered to be XVI. This structure is fully supported by its electron impact induced fragmentation as depicted in XVII;

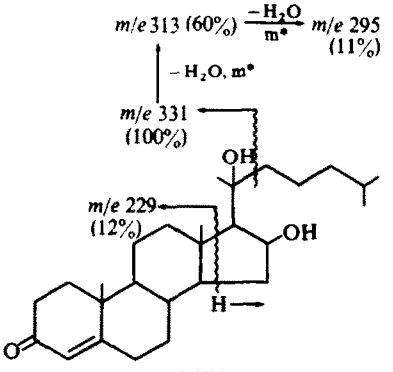
fragmentation similar to that shown in XIII (for guggulsterol-II) gives in this case an ion at *m/e* 270 (22%).

Biogenetic pattern

It has been demonstrated[28] that in mammalian tissues progesterone arises by the pathway: cholesterol → 20α-hydroxycholesterol → 20α,22S-dihydroxycholesterol → pregneneolone → progesterone. The catabolism of C_{27} precursor to C_{21} steroids in the plants is considered[29] to essentially follow the same route. The various steroids



XVI



XVII

now shown to co-occur in *Commiphora mukul* provide a satisfying biogenetic pattern fully consistent with the biosynthetic scheme. In this connection it will be pertinent to find out the C-20 stereochemistry in guggulsterol-II and guggulsterol-III and, the C-20, C-22 stereochemistry in guggulsterol-I. Due to the complexity of the gum-resin, it is inevitable that some other steroid components should have gone undetected, but it will be worthwhile from a biogenetic point of view, to look for components without oxygenation at C-16 and it is proposed to carry out this work. The occurrence in nature of 4,17(20)-pregnadiene-3,16-diones (I, II) is most interesting and a consideration of their genesis in nature offers several possibilities and if 16β-hydroxyprogesterone (guggulsterol-I → 16β-hydroxyprogesterone → *Z*- and *E*-guggulsterone) is an intermediate, its transformation into guggulsterones (I, II) calls for an interesting sequence of reactions.

EXPERIMENTAL

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

The silica gel for column chromatography was 100–200 mesh, was washed with hot distilled water till sulphate-free, dried and activated at 125–130° (6–8 hr) and then standardized.[30] $AgNO_3$-impregnated silica gel was made by the method of Gupta and Dev[31] and activated at 100–110° (4 hr). TLC was carried out on silica gel or silica gel–$AgNO_3$ (15% $AgNO_3$) layers (0·3 mm) containing 15% gypsum.

Following instruments were used for spectral data: Perkin–Elmer spectrophotometer, model 350 (UV); Perkin–Elmer Infracord, model 137E (IR); Varian Associates A-60 spectrometer (PMR; TMS as internal standard); CEC mass spectrometer, model 21-110B (Mass; 70 eV, direct inlet system).

Broad separation

The gum-resin was in the form of light to dark brown conglomerates of tears and was only slightly sticky

to touch and had a faint balsamic odour. The material was collected from Bhuj (Gujarat), India, during September 1969.*

The gum-resin (200 g) was repeatedly triturated with light petroleum (1 lit. × 5) to yield, after solvent removal, an extract (21·5 g, thick yellow liquid) and a residue. The residue was further triturated with EtOAc (500 ml × 6) to furnish, after solvent distillation, a dark brown gum (65 g) and an insoluble residue (114 g, off-white powder).

The EtOAc-insoluble residue has high ash content (~15%) which was found† to consist of oxides of Si, Ca, Al, Mg and Fe (chemical analysis) and traces of Ti, Cu and Na oxides (AC emission flame spectroscopy).

Separation of components of light petroleum fraction

The above light petroleum extract (39 g) was chromatographed on SiO_2-gel/IIb (114 cm × 4·5 cm) to effect broad separation.

TABLE 1. BROAD-CUT SEPARATION OF LIGHT PETROLEUM EXTRACT

Fr. 1	Light pet.	1 litre × 3	3·9 g, liquid
Fr. 2	C_6H_6	1 litre × 5	14·9 g, thick liquid
Fr. 3	Ether	1 litre × 4	19·0 g, brown gum
Fr. 4	MeOH	1 litre × 2	0·5 g, rejected

Diterpene hydrocarbon. Fr. 1 above, on TLC on $AgNO_3$–silica gel (solvent: 10% ether in light petroleum) showed the presence of at least 3 compounds of which one was major. Fr. 1 (15 g) was chromatographed on $AgNO_3$–silica gel (106 cm × 4 cm) while monitoring by TLC:

Fr. 1A	light pet.	250 ml × 8	1·94 g, mixture
	10% C_6H_6 in light pet.	250 ml × 6	
Fr. 1B	C_6H_6	250 ml × 2	1·93 g, mixture
Fr. 1C	C_6H_6	250 ml × 10	12·0 g, essentially single spot
	2% EtOAc in C_6H_6	250 ml × 5	
Fr. 1D	EtOAc	250 ml × 4	0·8 g, mixture

Fr. 1C (2·0 g) was rechromatographed on $AgNO_3$–silica gel (40 cm × 1·2 cm) using 10% ether in light petroleum as the eluent to furnish a TLC pure liquid (1·6 g), b.p. 150–152°/0·8 mm, n_D^{30} 1·5102, $[\alpha]_D$ −19·7° (*c*, 0·35%) (Found: C, 88·21; H, 12·11. $C_{20}H_{32}$ requires: C, 88·16; H, 11·84%).

Diterpene alcohol. Fraction 2 (Table 1) was found to be a mixture of at least 5 compounds, one of which was major (TLC; 30% ether in light petroleum). This mixture (5 g) was chromatographed on SiO_2-gel/IIa (100 cm × 4 cm) and followed up by TLC:

Fr. 2A	light pet.	1 lit. × 3	0·22 g, gum, mixture
	50% C_6H_6 in light pet.	500 ml × 6	
Fr. 2B	C_6H_6	250 ml × 1	0·29 g, gum, mixture
Fr. 2C	C_6H_6	200 ml × 3	3·35 g, gum, single
Fr. 2D	C_6H_6	250 ml × 5	0·67 g, gum, mixture
Fr. 2E	EtOH	250 ml × 4	0·32 g, gum, mixture

Fr. 2C (1·16 g) was crystallized from MeCN (1 ml) at ~ −10° and the solid (0·70 g) separated by inverse filtration and further recrystallized from MeCN, m.p. 37–38°, $[\alpha]_D$ +53° (*c*, 0·47%) (Found: C, 82·88; H, 11·81. $C_{20}H_{34}O$ requires: C, 82·69; H, 11·80%).

(+)-Sesamin. Fr. 3 (Table 1) was a very complex mixture by TLC (60% ether in light petroleum) and was

* The authors are grateful to Dr. C. K. Atal and Dr. C. Dwarakanath for the supply of raw material.

† The analysis was kindly carried out by Dr. P. R. Subbaraman and the authors are grateful to him for this help.

chromatographed on SiO_2-gel/IIb (124 cm × 4·5 cm) using increasing amounts of EtOAc in C_6H_6:

Fr. 3A	2% EtOAc in C_6H_6	500 ml × 3	2·11 g, gum, mixture
Fr. 3B	5% EtOAc in C_6H_6	250 ml × 6	2·64 g, gum, mixture
Fr. 3C	EtOAc in C_6H_6	250 ml × 7	3·28 g, gum, mixture
Fr. 3D	EtOAc in C_6H_6	250 ml × 14	4·52 g, gum, mixture
Fr. 3E	EtOAc in C_6H_6	250 ml × 8	1·15 g, gum, mixture
Fr. 3F	EtOAc in C_6H_6	250 ml × 12	1·60 g, gum, mixture
Fr. 3G	10% EtOAc in C_6H_6	250 ml × 5	2·92 g, gum, mixture
	25% EtOAc in C_6H_6	250 ml × 14	
Fr. 3H	EtOH	250 ml × 8	1·40 g, gum, mixture

Fr. 3A (2·11 g) was rechromatographed on SiO_2-gel/IIb (120 × 2 cm) and eluted with increasing amounts of ether in light petroleum. Fractions eluted with 20% ether in light petroleum were combined (0·56 g), dissolved in MeOH and chilled to furnish a colourless crystalline solid (160 mg), m.p. 120–123°, $[\alpha]_D$ +53·3° (*c*, 1·8%), identified as sesamin by comparison (m.m.p., IR) with an authentic sample (Lit.[15]: m.p. 123°, $[\alpha]_D$ 71°).

Cholesterol. Fr. 3B (2·64 g) was rechromatographed on SiO_2-gel/IIb (100 cm × 2 cm) as above and the material (1·56 g) eluted with 30% ether in light petroleum, on treatment with MeOH gave a solid (0·75 g), which after several recrystallizations from MeOH furnished white flakes m.p. 139–142°, identified as cholesterol by comparison (m.m.p., IR, PMR) with an authentic sample.

Z-Guggulsterone [4,17(20)-trans-*pregnadiene*-3,16-*dione*]. Fr. 3D (4·52 g), on treatment with ether and chilling gave a crystalline solid (1·44 g; m.p. 187–191°). Its mother liquor and Fr. 3E were combined (3·7 g) and chromatographed on SiO_2-gel/IIb (105 cm × 2·7 cm) using increasing amounts of ether in light petroleum as eluent with TLC monitoring (60% ether in light petroleum). The material (1·07 g), on treatment with ether as before furnished an additional quantity (0·38 g; m.p. 182–189°) of the same solid (TLC). The combined solids were recrystallized from acetone to furnish colourless prisms (1·23 g), m.p. 192–193°, $[\alpha]_D$ −77·1° (*c*, 2·07%); λ_{max}^{EtOH} 241 nm (ε, 27,100). IR (Nujol): C=O 1720, 1675 cm^{-1}; C=C 1650, 1620 cm^{-1}. PMR ($CDCl_3$): C-18 Me (3H, s, 0·97 ppm), C-19 Me (3H, s, 1·23 ppm), C-21 Me (3H, d, 2·08 ppm, J = 7 Hz), C-4H (1H, b s, 5·75 ppm) and C-20H (1H, q, 5·73 ppm, J = 7 Hz). Mass spectrum: important ions at *m/e* 312 (M^+, 41%), 298 (24%), 297 (100%), 135 (13%), 121 (8%), 105 (9%), 93 (10%), 91 (18%), 79 (14%), 77 (12%), 55 (9%) and 53 (9%). (Found: C, 80·61; H, 9·01. $C_{21}H_{28}O_2$ requires: C, 80·73; H, 9·03%) [Lit.[18]: m.p. 188–190°; $[\alpha]_D$ −61°; λ_{max} 241 nm (ε 25,000); IR; PMR].

A soln of *Z*-guggulsterone (0·30 g) in THF (20 ml) was added dropwise to a soln of Li (650 mg) in liquid NH_3 (180 ml) during 10 min with continuous stirring. After stirring for another 90 min excess of Li was destroyed by NH_4Cl (13 g), NH_3 evaporated, water (75 ml) added and the product taken up in $CHCl_3$ (50 ml × 3). Solvent removal yielded a gum (0·31 g) which was dissolved in pyridine (3·5 ml) and oxidized with Sarett reagent (CrO_3, 320 mg; pyridine, 3·5 ml) for 16 hr at ~25° to furnish after usual work up the crude saturated ketone (268 mg) which was purified by PLC (25% EtOAc in C_6H_6) to give after crystallization from aq. MeOH, 5α-pregnan-3,16-dione (146 mg), m.p. 125–127°; IR ($CHCl_3$): C=O 1740, 1715 cm^{-1} (Lit.[19]: m.p. 124–128°).

E-*Guggulsterone* [4,17(20)-cis-*pregnadiene*-3,16-*dione*]. Fr. 3F (1·60 g) on treatment with ether and chilling furnished a solid (0·25 g) which, on further recrystallization (C_6H_6-light petroleum) at −10° gave colourless needles (TLC pure; solvent: 60% ether in light petroleum), m.p. 168–170°, $[\alpha]_D$ −28·4° (*c*, 2·11%), λ_{max}^{EtOH}, 241 nm (ε, 22,220). IR (Nujol): C=O 1720, 1670 cm^{-1}; C=C 1650, 1620 cm^{-1}. PMR (CCl_4): C-18 Me (3H, s, 1·07 ppm), C-19 Me (3H, s, 1·23 ppm), C-21 Me (3H, d, 1·85 ppm, J = 7 Hz), C-4H (1H, b s, 5·67 ppm) and C-20H (1H, q, 6·45 ppm, J = 7 Hz). Mass spectrum: important ions at *m/e* 312 (M^+ 100%), 298 (10%), 297 (41%), 271 (10%), 270 (42%), 255 (13%), 227 (8%) and 214 (8%). (Found: C, 80·71; H, 9·12. $C_{21}H_{28}O_2$ requires: C, 80·73; H, 9·03%). [Lit.[18]: m.p. 170–171·5°; $[\alpha]_D$ −30°; λ_{max} 241 nm (ε, 27,600); IR; PMR].

Saponification and chromatography of EtOAc *extract*

The EtOAc extract (100 g) was refluxed (N_2) with 10% aq. methanolic KOH (2·0 lit.) for 3 hr. Water (500 ml) was added and MeOH (~400 ml) removed; after further dilution with H_2O (1·51 l) and usual work up with ether, acidic material (41·5 g) and non-saponifiable portion (56 g, reddish gum) were obtained. The latter was chromatographed on SiO_2-gel/IIb (120 cm × 7 cm) using increasing amounts of EtOAc

in C_6H_6 as the eluent and with TLC monitoring [solvents: (a) 60% ether in light petroleum; (b) 10% MeOH in C_6H_6]:

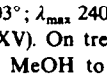
Fr. 1	C_6H_6	500 ml × 10	1·30 g, gum, mixture
Fr. 2	2% EtOAc in C_6H_6	500 ml × 5	3·25 g, gum, mixture
Fr. 3	2–5% EtOAc in C_6H_6	500 ml × 9	2·04 g, gum, mixture
Fr. 4	10% EtOAc in C_6H_6	500 ml × 8	2·11 g, gummy crystalline mixture
Fr. 5	25% EtOAc in C_6H_6	500 ml × 5	0·91 g, gum, mixture
Fr. 6	25% EtOAc in C_6H_6	500 ml × 2	3·75 g, gummy crystalline mixture
Fr. 7	25% EtOAc in C_6H_6	500 ml × 2	2·19 g, gum, mixture
Fr. 8	25–50% EtOAc in C_6H_6	500 ml × 4	2·91 g, gummy crystalline mixture
Fr. 9	50% EtOAc in C_6H_6	500 ml × 4	4·77 g, gum, mixture
Fr. 10	50% EtOAc in C_6H_6	500 ml × 4	1·49 g, gummy crystalline mixture
Fr. 11	50% EtOAc in C_6H_6	500 ml × 3	1·54 g, gum, mixture
Fr. 12	50% EtOAc in C_6H_6	500 ml × 8	2·65 g, gummy crystalline mixture
Fr. 13	EtOAc	500 ml × 4	3·53 g, gum, mixture
Fr. 14	2–50% MeOH in EtOAc	500 ml × 28	16·39 g, gummy solid
Fr. 15	MeOH	500 ml × 4	2·25 g, rejected

Fr. 6 (3·75 g) was chromatographed on SiO_2–gel/IIb (112 cm × 2·5 cm) and eluted with increasing amounts of EtOAc in C_6H_6. Fractions eluted with 25% EtOAc in C_6H_6 were combined and crystallized from acetone to furnish *Z*-guggulsterone (0·566 g), m.p. 189–191°.

Fr. 8 (2·91 g) was similarly chromatographed on SiO_2–gel/IIb (94 cm × 2·5 cm). The fractions eluted with 25% EtOAc in C_6H_6 were combined, treated with ether and chilled to furnish *E*-guggulsterone (0·39 g) which on recrystallization from C_6H_6–light petroleum had m.p. 168–170°.

Guggulsterol-I (VIII). Fr. 12 (2·60 g) was chromatographed on SiO_2–gel/IIb (90 cm × 2·5 cm) and eluted with increasing proportions of EtOAc in C_6H_6. Fractions eluted with 50% EtOAc in C_6H_6 were combined (1·9 g), treated with MeCN (5 ml) and chilled to furnish a solid (m.p. 221–226°, 750 mg) which on recrystallization from aq. MeOH gave colourless crystals, m.p. 225–228°, $[\alpha]_D$ +77·6° (*c*, 2·01%). (Found: C, 75·28; H, 10·63. $C_{27}H_{44}O_4$ requires: C, 74·95; H, 10·25%).

A soln of guggulsterol-I (305 mg) in MeOH (15 ml) was treated with a soln of $NaIO_4$ (311 mg) in water (3 ml) and MeOH (12 ml) and kept in the dark (36 hr). The mixture was diluted with water (75 ml) and extracted with CH_2Cl_2 and the crude product chromatographed on SiO_2–gel/IIa (39 cm × 1·2 cm). The material eluted with CH_2Cl_2 (150 ml) was, after solvent removal, treated with 2,4-dinitrophenylhydrazine (H_2SO_4 method) diluted with water (10 ml) and the derivative extracted with $CHCl_3$. PLC (solvent: 25% light petroleum in C_6H_6) of this product gave 2,4-DNP of *iso*caproic aldehyde (top cut, 12 mg) m.p. 88–91°; m.m.p. with authentic sample (m.p. 88–90°) was undepressed and their spectra (IR, PMR) were identical. The chromatography fraction (165 mg) eluted with 2% MeOH in CH_2Cl_2 (50 ml) was essentially pure by TLC (10% MeOH in C_6H_6): this was further purified by PLC and the product recrystallized from acetone–light petroleum to give 16β-hydroxyprogesterone (100 mg), m.p. 203–204°, $[\alpha]_D$ +207·5° (*c*, 1·6%): λ_{max}^{EtOH} 240 nm (ε, 17,600). IR ($CHCl_3$): OH 3420, 1040 cm^{-1}; C=O 1705, 1665 cm^{-1}; C=C 1620 cm^{-1}. PMR ($CDCl_3$): 2 quaternary Me's (6H, s, 1·2 ppm), $C\underline{H}_3C{=}O$ (3H, s, 2·2 ppm), $C\underline{H}OH$ (1H, m, 4·55 ppm),



(1H, s, 5·7 ppm). (Found: C, 76·61; H, 9·19. $C_{21}H_{30}O_3$ requires: C, 76·32; H, 9·15%).

(Lit.[24]: m.p. 202–203°; λ_{max} 240 nm, ε, 16,500; IR; Mass).

Guggulsterol-II (XV). On treatment with MeCN fr. 10 (1·49 g) deposited a solid (70 mg) which was recrystallized from MeOH to furnish colourless crystals m.p. 231–233° (evacuated sealed capillary), $[\alpha]_D$ −42·3° (*c*, 0·22%). (Found: C, 77·32; H, 10·83. $C_{27}H_{46}O_3$ requires: C, 77·46; H, 11·08%). Its *diacetate* (Ac_2O, pyridine, room temp/12 hr) was obtained as snow-white flakes, m.p. 179–181° (MeOH). (Found: C, 73·35; H, 9·96. $C_{31}H_{50}O_5$ requires: C, 74·06; H, 10·03%).

A stirred slurry of guggulsterol-II (127 mg) in ether (100 ml) was treated with Brown's reagent[25] ($Na_2Cr_2O_7$ 1 g, conc. H_2SO_4 0·75 ml made up to 5 ml with H_2O) in portions, at 25–30°. After 2 hr, the faint-orange soln was treated with a few drops of MeOH and worked up to furnish a gum (61 mg) which was purified by PLC (solvent: 5% MeOH in C_6H_6); the required ketonic compound (highest R_f, 16 mg) was

now TLC pure but still remained a gum. PMR (CCl_4): $\underline{Me}_2CH$— (6H, d, 0·88 ppm, J = 6 Hz), 3 quaternary Me's (6H, s, 1·2 ppm; 3H, s, 1·3 ppm), O=C—C$\underline{H}$=C (1H, s, 6·1 ppm).

Guggulsterol-III (XVI). Fr. 9 (4·77 g) was chromatographed on SiO_2-gel/IIb (90 cm × 3 cm), the fractions eluted with 25% EtOAc in C_6H_6 were combined and treated with MeCN (~5 ml). The separated solid (273 mg, m.p. 160–165°) was a mixture (TLC) of two compounds one of which was guggulsterol-II. The other compound (95 mg) was isolated by PLC (10% MeOH in C_6H_6) and recrystallized from acetone to afford colourless silky needles (42 mg), m.p. 181–183°, $[\alpha]_D$ +75·3° (*c*, 0·17%). (Found: C, 77·78; H, 10·87. $C_{27}H_{44}O_3$ requires: C, 77·83; H, 10·65%).

Partial synthesis of guggulsterol-II

Pseudodiosgenin diacetate[26] (5·0 g, m.p. 94–97°) in gl. AcOH (100 ml), cooled to 15° in ice–water bath, was oxidized[27] with a soln of CrO_3 (3·5 g) in H_2O (3·5 ml) and AcOH (10 ml). The temp rose to 28° and after 45 min it was poured into water (300 ml) and the neutral product isolated with ether to furnish a gum (4·48 g); TLC (15% EtOAc in C_6H_6) showed it to be a complex mixture. A TLC pure fraction (1·51 g) was isolated by IDCC[32] on SiO_2-gel (25 cm × 9·4 cm; solvent 15% EtOAc in C_6H_6) and recrystallized from MeOH to furnish colourless crystals of ketoester XIV (817 mg), m.p. 82–85° (Lit.[27]: m.p. 84–86°).

Mg turnings (632 mg) in dry ether (20 ml) were stirred and treated with isohexyl bromide[33] (3·33 g) in dry ether (30 ml) in a 3-necked flask equipped with a dropping funnel, stirrer and condenser and also provided with a gas-inlet tube for passing O_2-free dry N_2. After 20 min stirring at room temp the mixture was gently refluxed (3 hr) on a waterbath. To this Grignard reagent (estimated:[34] 60%) was added the keto ester XIV (521 mg) in dry ether (10 ml) and refluxed for 1 hr with stirring. Benzene[35] (50 ml) was added and the ether distilled off and the resulting product further refluxed for 14 hr. The complex was decomposed with NH_4Cl aq., C_6H_6 layer separated, aq. portion extracted with ether and the combined organic portions washed, dried and evaporated to furnish a material, which was a complex mixture (TLC: solvent 10% MeOH in C_6H_6), but had a component with the same R_f as guggulsterol-II. The products from two such experiments were combined, hydrolyzed with aq. ethanolic KOH (10%, 42 ml) at reflux (4 hr, N_2) and the crude material (2·0 g) treated with C_6H_6–ether when cryst. guggulsterol-II (129 mg) separated. The crude material from the filtrate was chromatographed on SiO_2-gel/IIb (110 cm × 2·5 cm) and eluted with C_6H_6 followed by increasing amounts of EtOAc in C_6H_6. The fraction eluted with 25% EtOAc in C_6H_6 on treatment with MeCN yielded an additional quantity of guggulsterol-II (34 mg). The combined products were recrystallized from MeOH to furnish pure guggulsterol-II (68 mg), m.p. 231–234° (evacuated sealed capillary), m.m.p. with natural sample (m.p. 231–233°) was undepressed; $[\alpha]_D$ −45·2° (*c*, 0·2%). *Diacetate*, m.p. 177–179°; m.m.p. with authentic guggulsterol-II diacetate (m.p. 179–181°) was undepressed.

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