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CHEMISTRY OF AYURVEDIC CRUDE DRUGS—V†

GUGGULU (RESIN FROM *COMMIPHORA MUKUL*)—5 SOME NEW STEROIDAL COMPONENTS AND, STEREOCHEMISTRY OF GUGGULSTEROL-I AT C-20 AND C-22‡

ASHOK G. BAJAJ§ and SUKH DEV*
Malti-Chem Research Centre, Nandesari, Vadodara, India

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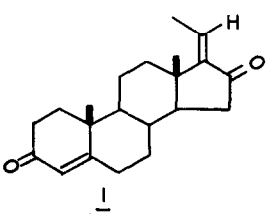
Abstract—20α-hydroxy-4-pregnen-3-one (**5**), 20β-hydroxy-4-pregnen-3-one (**6**), 16β-hydroxy-4,17(20)*Z*-pregnadien-3-one (**4**) and 16α-hydroxy-4-pregnen-3-one (**10**) have been isolated as new steroidal components of the gum-resin from *Commiphora mukul*. A simple procedure for the synthesis of **4** is described. Chirality at C-20, C-22 in guggulsterol-I (**3**) has been clarified.

Isolation of several steroidal constituents from the gum-resin of *Commiphora mukul* (Hook, ex Stocks) Engl. (Syn. *Balsamodendron mukul* Hook, ex Stocks) has been reported[1] and one of these (Z-guggulsterone, **2**) has since been shown to possess significant hypolipaemic acitivity.[2] Since the hypolipaemic acitivity of the total fraction from which Z-guggulsterone was isolated, was far in excess of its Z-guggulsterone (**2**) or the closely-related E-guggulsterone (**1**) content, it was decided to look for other minor constituents[3] which might have a more pronounced activity or might act synergistically. With this aim in view, the gum-resin has been fractionated in a more systematic manner: the neutral ethyl acetate soluble fraction (~40% of gum-resin; *vide* Experimental) which carries the hypolipaemic acitivty, was separated, with the help of semicarbazide-silica gel,[4] into ketonic (12%) and non-ketonic fractions. A detailed analysis of the ketonic fraction which accounts for the bulk of the activity, forms the subject of the present investigation. Also, the hitherto unclarified stereochemistry at C-20 and C-22 in guggulsterol-I, has been deduced as depicted in **3**.

TLC, as well as HPLC showed the ketonic fraction to be highly complex with at least 23 components, encompassing a wide range of polarity. The ketonic fraction was, therefore, broadly separated into material less polar than E- and Z-guggulsterones (~7%), E- and Z-guggulsterones (~43%) and a more polar cut (~50%).

A detailed chromatographic analysis of the less polar cut, after saponification,[6] led to the isolation of four compounds, all C_{21} steroids. Three of these (**4–6**) are known and were recognised from their spectral data (MS, UV, IR, ^{1}H NMR) and the identity confirmed by preparing authentic samples from 16-dehydro-pregnenolone acteate (16-DPA, **11**). Whereas samples of **5** and **6** were obtained by known methods,[7,8] a simpler procedure for the synthesis of **4**, as compared to the one already known,[9] was adopted and is discussed in the sequel. Furthermore, though all these three compounds have been described in the literature, only **5** and **6** have been isolated from natural sources;[10,11] compound **4** is being reported to occur in nature for the first time and the trivial name, *Z-guggulsterol*, is assigned to it.



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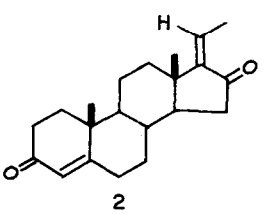

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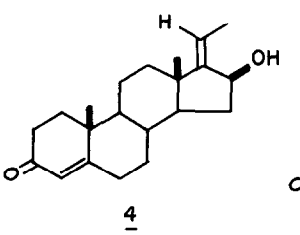

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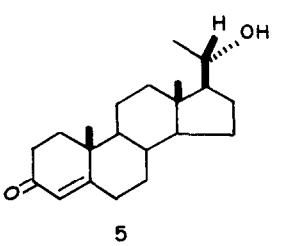

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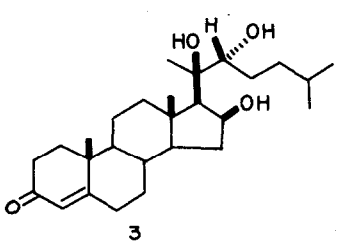

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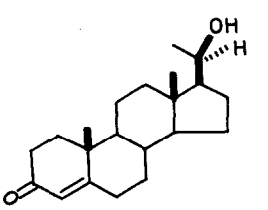

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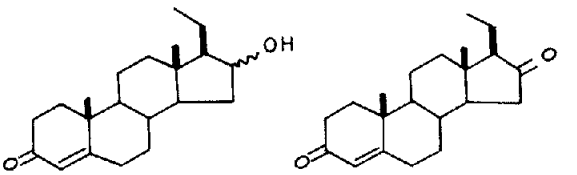
‡MRC Communication No. 32.
§Present address: Sekhsaria Chemicals Ltd., Dombivili, Thana, Bombay, India.

The fourth compound, which is a new naturally occurring C_{21} steroid, has been designated guggulsterol-VI.[12] From its high-resolution mass spectrum[13] (M^+, *m/e* 316.2407), the molecular formula of the compound was

deduced to be $C_{21}H_{32}O_2$. The compound shows the following structural features: two tertiary Me's (^{1}H NMR: two 3H singlets at 0.67, and 1.20 ppm), one CH_3–CH_2– (^{1}H NMR: 3H, t, 1.03 ppm, J = 6 Hz), CHOH (IR: 3445, 1100 cm^{-1}. ^{1}H NMR: 1H, ill-defined m, 4.03 ppm), and –CO–CH=C– (λ_{max}^{EtOH} 241 nm, ϵ 16,900. IR: 1660, 1610 cm^{-1}. ^{1}H NMR: 1H, s, 5.79 ppm). From the molecular formula and functionality, it is obvious that the compound is tetracarbocyclic and, from the nature of the ^{1}H NMR Me signals,[14] and previous knowledge[1] on the chemistry of the gum-resin, a steroid structure, such as 7, appeared most plausible. This contention could be confirmed and further refined as follows.

Hydrogenation of Z-guggulsternone (2; containing some 5% of E-guggulsterone, 1) on 2% Pd–$SrCO_3$, yielded 8 (λ_{max}^{EtOH} 242 nm, ϵ 16,500. IR (CCl_4): C=O 1742, 1678 cm^{-1}; C=C 1618 cm^{-1}. ^{1}H NMR: CO–CH=C–, 1H, s, 5.74 ppm) though in a poor yield; the β-configuration of the C-17 Et group follows from the anticipated hydrogenation from the α-face, for which there is ample precedence.[9,15] The product (8) was next reduced with LAH and then exposed to active MnO_2 to yield two epimeric alcohols (9, 10) which were separated by chromatography. The major product (~80%; m.p. 185–187°) is considered to be the β-epimer (9), as LAH reduction of similar 16-keto steroids is known[16] to furnish a preponderance of the 16β-epimer. The minor product was found to be identical ($[\alpha]_D$, m.p., mixed m.p., UV, IR, ^{1}H NMR and MS) with guggulsterol-VI, and this further defines the C-16 configuration as shown in 10.

The more polar fraction, eluted after E and Z-guggulsterones, on HPLC, failed to give any additional pure compounds, besides guggulsterol-I and guggulsterol-III, already reported.[1]



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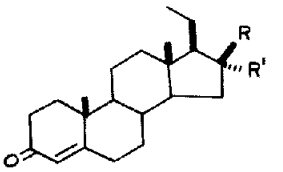
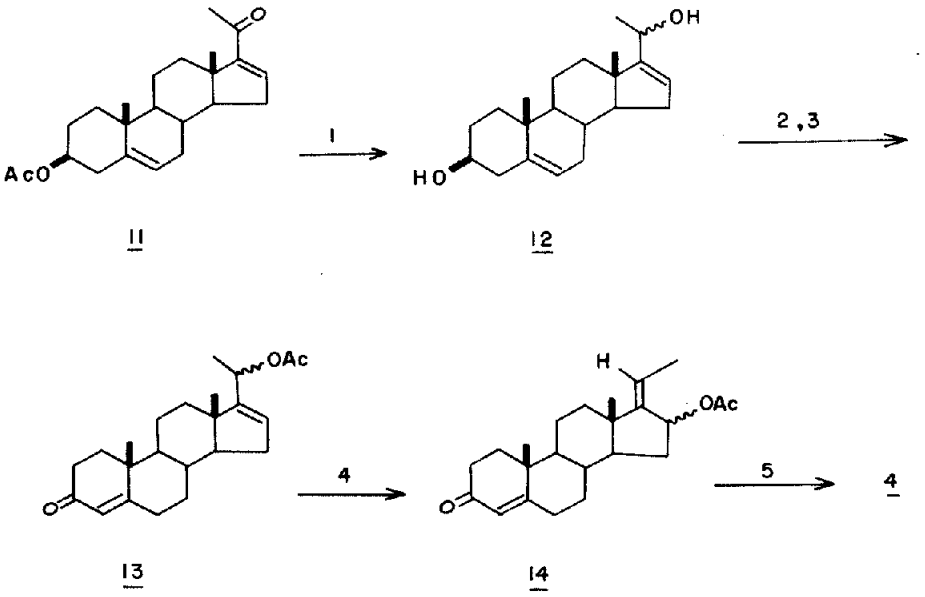


9 : R = OH, R' = H
10 : R = H, R' = OH

Synthesis of Z-guggulsterol (4). The sequence of reactions successfully adopted for the preparation of 4 from 16-DPA (11) is depicted in Fig. 1. LAH reduction of 11, carried out in inverse fashion, furnished 12 (epimeric mixture) quantitatively, in contrast to ~60% yields reported earlier.[17] Selective acetylation of 12 at C-20 was achieved by dry acetic acid containing catalytic amount of p-toluenesulphonic acid (p-TSA), and the product subjected to Oppenauer oxidation to furnish 13 (epimeric mixture). Allylic rearrangement of 13 gave 14, which had been prepared earlier[9] by a rather circuitous route. Alkali



11 12 13 14

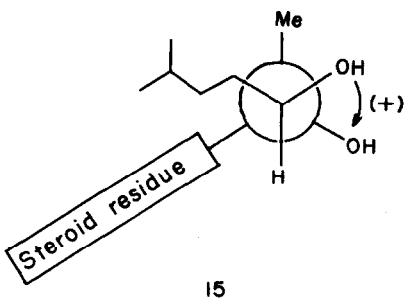
Reagents: 1. LAH 2. AcOH, p-TSA
3. $(t\text{-BuO})_3$ Al, acetone
4. Ac_2O, AcOH, p-TSA
5. 5% KOH – MeOH

Fig. 1. Synthesis of Z-guggulsterol.

saponification of **14** yielded a product, major in **4**, which was separated and found to be identical in all respects (m.p., mixed m.p., $[\alpha]_D$, IR, ^{1}H NMR, MS) with the naturally occurring compound. It may be noted that allylic rearrangement of type (**13**→**14**) is known to generate only Z-olefins with 16β-configuration being preferred, and these consequences are independent of the C-20 configuration.[9]

C-20, C-22 *Configuration in guggulsterol-I*

Previous work[1] had led to the structure elucidation of guggulsterol-I, except for the chirality at C-20 and C-22. In the first instance, Nakanishi's induced CD method,[18] but by using Eu(fod)$_3$ as the complexion agent,[19,20] was applied in resolving this problem. With an approx. 1:1 ratio of guggulsterol and Eu(fod)$_3$ in $CHCl_3$, the solution shows an induced split Cotton effect, the longer wavelength extremum (302.5 nm) of which is positive ($\Delta\epsilon = +2.17°$), indicating[18,19] (+)-chirality (**15**) of the substrate. This, then leads to (20S, 22S) configuration in guggulsterol-I. However, this assignment is not consistent with certain biogenetic considerations. It is well established that biological hydroxylations (–$\overset{|}{C}$H→–$\overset{|}{C}$–OH) occur with retention of configuration,[21] and on this consideration, one would have expected 20R chirality in guggulsterol-I, after making the very reasonable assumption that guggulsterol-I arises in plant, from cholesterol (**18**) (see following section). In view of this discrepancy, stereochemistry of guggulsterol-I was next investigated by X-ray crystallographic analysis,[22] which revealed a (20R, 22R) configuration (**3**), fully consistent with the biogenetic reasoning. It is conceivable that the induced CD method failed, because of possible interference from the C-16 OH.



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With the clarification of C-20, C-22 chirality in guggulsterol-I (**3**), and making the reasonable assumption that guggulsterol-I, -II' and -III' arise by suitable sequential transformations, the absolute configuration at C-20 in both guggulsterol-II (**16**) and guggulsterol-III (**17**) can be considered to be the same as in guggulsterol-I (**3**). (Absolute Sterochemistry Biogenetic Rule[23]).

Biogenetic pattern

There is sufficient evidence that both in mammalian tissues[24] and in plants,[25] the catabolism of cholesterol (**18**) proceeds by either of the two major pathways shown in Fig. 2. The steroids from *guggulu* provide a unique example of occurrence of cholesterol and each of the key intermediates (according to pathway a, but with additional hydroxylation at C-16) in such a catabolic sequence in the same plant tissue: Cholesterol (**18**)→ → guggulsterol-II (**16**)→ → guggulsterol-I (**3**)→ → Z-guggulsterol (**4**)→ Z-guggulsterone (**2**). The isolation of C_{21} steroids without oxygenation at C-16 (e.g. **5**, **6**) would suggest that, usual conversion of cholesterol into C_{21} steroids, without oxygenation at C-16 is another pathway operating in the plant. The occurrence of guggulsterol-VI (**10**), which has an Et group[26] at C-17 and an α-OH at C-16, probably reflects further transformation of guggulsterones (**1**, **2**).

EXPERIMENTAL

All m.ps are uncorrected. Light petroleum refers to the fraction, b.p. 60–80°. Optical rotations were measured in $CHCl_3$ on a Schmidt + Haensch electronic polarimeter model Polatronic I.

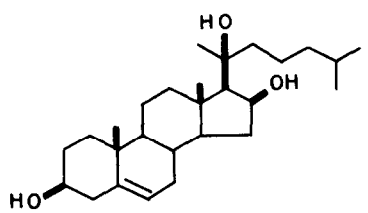
Silica gel for column chromatography (–100, +200 mesh) was activated at 125–130° (6–8 h) and then standardised.[27] Alumina (–100, +250 mesh) was made neutral (HNO_3 method[28]), activated at 400° (8–10 h) and then graded according to Brockmann.[29] TLC was carried out on layers (0.3 mm) of silica gel or silica gel-15% $AgNO_3$ containing gypsum (15%) as binder, and activated at 100–110° (1 h). All chromatographic separations were monitored by TLC.

The following instruments were used for spectral/anaytical data: Perkin–Elmer model 267 IR spectrophotometer; Perkin–Elmer model 402 UV spectrophotometer; Perkin–Elmer model R32 (90 MHz) NMR spectrometer; Varian Mat CH7 mass spectrometer (70 eV, direct inlet system); DuPont 848 liquid chromatograph. All ^{1}H NMR spectra were recorded in $CDCl_3$ (unless stated otherwise) with TMS as internal reference; signals are reported in ppm (δ). While citing ^{1}H NMR data, following abbreviations have been used: S (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and b (broad). While summarising mass spectral data, besides the molecular ion, ten most abundant ions (above *m/e* 50) are given with their relative intensities.

Neutral, ketonic fraction of gum-resin. 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. This material was from a stock collected several years ago from Bhuj (Gujarat), and had been carefully preserved.

The gum-resin (1000 g) was covered with EtOAc (2 l.) and after letting it stand at room temp (30–35°) for 6 h, the extract was withdrawn and the operation repeated another 6 times, now using 1 l. of EtOAc each time. The combined EtOAc extracts were freed of solvent (~50°/200→20 mm) to furnish the extract (446 g) as a dark brown gum. The EtOAc insoluble material (550 g) was an off-white friable solid.

The EtOAc extract (250 g) was taken up in EtOAc (1 l.) and extracted with 3N HCl aq (125 ml × 2). The acid extract on basification with NH_3aq and usual work-up (EtOAc) gave *basic material* (0.8 g) significantly contaminated with acetamide. The



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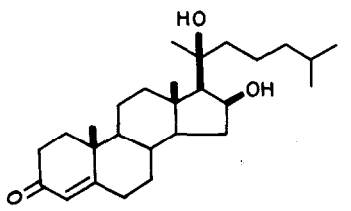

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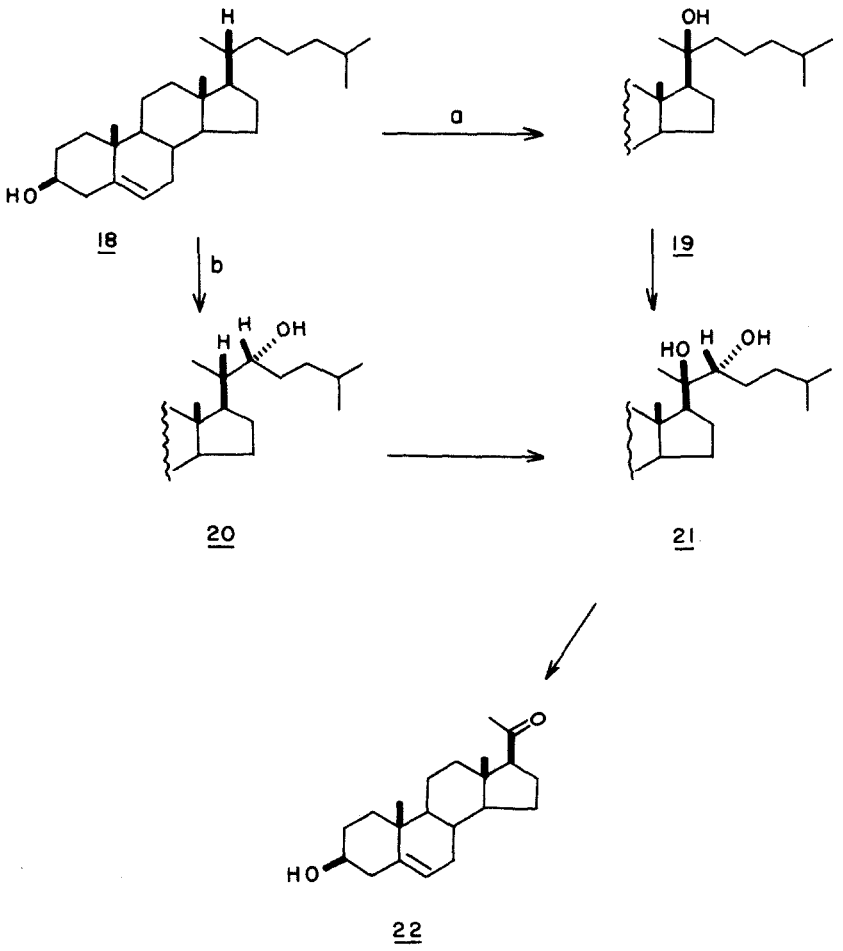

Fig. 2. Catabolism of cholesterol to pregnenolone.

EtOAc layer was next extracted with 10% Na_2CO_3 aq (500 ml × 1; 250 ml × 7) and the carbonate extract acidified (25% v/v H_3PO_4 aq, ~ 1350 ml) to get (EtOAc extraction) ~ 10 g of *acidic fraction* (dark brown gum). The neutral EtOAc layer was finally washed with brine (250 ml × 2) and freed of solvent to furnish bulk of the material as the neutral fraction (215 g, dark brown gum).

A mixture of the neutral fraction (725 g), 10% semicarbazide-on-silica gel[4] (750 g) and toluene (1 l.) was stirred and heated (60–62°) for 14 h, then cooled (room temp) and filtered and the silica gel thoroughly washed with toluene (200 ml + 3). Solvent removal furnished *non-carbonyl material* (~ 635 g). The above washed silica gel, oxalic acid aq (10% w/v, 750 ml) and toluene (750 ml) were stirred and refluxed (2.5 h), after which it was cooled and filtered. Silica gel was washed with EtOAc (250 ml × 2). The combined solvent extract was washed with water and brine and freed of solvent to give the required *ketonic fraction* (90 g, dark brown gum). Both TLC (SiO_2 gel; solvent, 25% EtOAc in C_6H_6) and HPLC (Zorbax ODS column, 4.6 mm × 25 cm; eluting solvent, 15% H_2O in MeOH; solvent flow, 0.6 ml/min; pump pressure, 1000 psi; UV detector, 254 nm) indicated this ketonic fraction to be extremely complex with no less than twenty threee components.

Separation of neutral ketonic fraction. The ketonic fraction (10 g) was chomatographed on SiO_2-gel/IIb (70 cm + 4.5 cm) and eluted with increasing amount of EtOAc in C_6H_6 to get three broad cuts: (1) 0–10% EtOAc in C_6H_6, 250 ml × 17, 0.6281 g of a yellow gum, (2) 15–25% EtOAc in C_6H_6, 250 ml × 15, 4.2 g of a mix. of Z- and E-guggulsterones, (3) 50% EtOAc in C_6H_6, 250 ml × 6, and 250 ml × 2 of MeOH, total 4.9 g of a partly crystalline gum.

Fraction (1) above (1.45 g) was rechromatographed on SiO_2 gel/IIb (78 cm × 2 cm) and the fractions eluted with C_6H_6 (A, 50 ml × 6, 0.1967 g, gum), 2% EtOAc in C_6H_6 (B, 50 ml × 3, 0.3260 g, gum), 5–10% EtOAc in C_6H_6 (C, 50 ml × 8, 0.7417 g, gum) and 12–25% EtOAc in C_6H_6 (D, 50 ml × 14, 0.1875 g, gum). Fraction B (0.4 g) was saponified (10% KOH methanolic, 2 h, reflux) and the non-saponifiable part (0.3795 g, yellow gum) systematically rechromatographed on SiO_2 gel/IIb (73 cm × 1 cm) to get fractions: B_1 (C_6H_6, 1–3% EtOAc in C_6H_6, 50 ml × 8 and 25 ml × 34, 0.0935 g, gum, mixture), B_2 (5% EtOAc in C_6H_6, 25 ml × 2, 0.0312 g, TLC pure), B_3 (5% EtOAc in C_6H_6, 25 ml × 10, 0.1021 g, TLC pure), B_4 (5% EtOAc in C_6H_6, 25 ml × 6, 0.0344 g, TLC pure), B_5 (7% EtOAc in C_6H_6, 25 ml × 14, 0.0120 g, TLC pure) and B_6 (10–20% EtOAc in C_6H_6, 25 ml × 20, 0.0959 g, gum, mixture).

Z-guggulsterol (**4**). Fraction B_2 on crystallization from acetone-hexane furnished colourless prisms, m.p. 169–172°, $[\alpha]_D$ + 137.6° (c, 1.6%). λ_{max}^{EtOH} 242 nm (ε, 15400). IR ($CHCl_3$): 3590, 3440, 1660, 1610, 1370, 1356, 1327, 1270, 1015, and 860 cm^{-1}. ^1H NMR: C-Me's (3H singlets at 0.98 and 1.21 ppm), MeCH=C (3H, d, 1.74 ppm, J = 7 Hz), CHOH (1H, t, 4.76 ppm, J = 7.5 Hz), MeCH=C (1H, dxq, 5.29 ppm, J_1 = 7Hz, J_2 = 2Hz), O=C-CH=C (1H, s, 5.74 ppm). MS: *m/e* 314.2243 (M^+, calcd. for $C_{21}H_{30}O_2$, 314.224568); *m/e* 314 (100%), 299 (70%), 296 (29%), 231 (50%), 124 (23%), 123 (26%), 122 (22%), 119 (24%), 107 (25%), 85 (28%), 84 (43%). (Lit.[9]: m.p. 172–175°, $[\alpha]_D$ + 141.5°; UV, IR, ^1H NMR).

(20*S*)-20-*hydroxy*-4-*pregnen*-3-*one* (**5**). Fraction B_3 was recrystallised from EtOAc-hexane to give colourless, flat needles, m.p. 160–161°, $[\alpha]_D$ + 96.54° (c, 2%). λ_{max}^{EtOH} 242 nm (ε, 15130). IR ($CHCl_3$): 3600, 3440, 1660, 1615, 1380, 950 and 870 cm^{-1}. ^1H

NMR: C-Me's (3H singlets at 0.73 and 1.20 ppm). 4Me–CH (3H, d, 1.21 ppm, $J = 6.5$ Hz), CHOH (1H, m, 3.75 ppm), O=C–CH=C (1H, s, 5.75 ppm). MS: *m/e* 316.2400 (m^+ calc. for $C_{21}H_{32}O_2$, 316.240217); *m/e* 316 (73%), 298 (19%), 274 (23%), 231 (22%), 230 (26%), 191 (26%), 175 (27%), 149 (28%), 147 (18%), 135 (16%), 124 (100%). (Lit.[7]: m.p. 161–162°, $[\alpha]_D + 98.6°$; ^1H NMR).

(20R)-20-*hydroxy*-4-*pregnen*-3-*one* (**6**). Recrystallization of B_4 from EtOAc yielded minute prisms, m.p. 168–170°, $[\alpha]_D + 88.0°$ (c, 2.5%). λ_{max}^{EtOH} 242 nm (ϵ, 14850). IR ($CHCl_3$): 3595, 3440, 1660, 1615, 1380, 1338, 1280, 1100, 965, 880 and 868 cm^{-1}. ^1H NMR: C-Me's (3H singlets at 0.81 and 1.21 ppm), Me–CH (3H, d, 1.51 ppm, $J = 6.5$ Hz), CHOH (1H, m, 3.78 ppm), O=C–CH=C (1H, s, 5.78 ppm). MS: *m/e* 316.2400 (M^+, Calc. for $C_{21}H_{32}O_2$, 316.240217); *m/e* 316 (88%), 301 (6%), 298 (14%), 274 (30%), 231 (18%), 229 (15%), 193 (10%), 175 (30%), 149 (30%), 124 (100%). (Lit.[8,11] m.p. 169–171°, $[\alpha]_D + 84°$; IR, ^1H NMR).

Guggulsterol-VI (**7**). Fraction B_5, on recrystallization from acetone afforded colourless needles, m.p. 197–199.5°, $[\alpha]_D +$ 114.0° (c, 0.4%). IR ($CHCl_3$): 3445, 1660, 1610, 1378, 1275, 1100, 950 and 862 cm^{-1}. UV and ^1H NMR, see text. MS: *m/e* 316.2407 (M^+, Calc. for $C_{21}H_{32}O_2$, 316.240217); *m/e* 316 (10%), 279 (39%), 167 (61%), 149 (100%), 113 (17%), 112 (16%), 83 (8%), 71 (21%), 70 (19%), 57 (21%). (Found: C, 79.30; H, 10.25. $C_{21}H_{32}O_2$ requires: C, 79.69; H, 10.20%.)

Synthesis of Z-guggulsterol (**4**)

5,16-*pregnadiene*-3β,20α-*and* 3β,20β-*diol* (**12**). To a cooled (0°) soln of 16-DPA (3.0 g, 0.0084 mole) in dry ether (100 ml) was added, with stirring, a suspension of LAH (0.540 g) in dry ether (30 ml) during 20 min, while maintaining the temp ~0°. After stirring at 0° for another 2 h, the R mixture was worked up in the usual manner (EtOAc and conc Na_2SO_4 aq) to afford a product (2.6107 g), which on one crystallization from acetone gave colourless needles (1:1 molecular complex of C-20 *epimers*[17]; also clear from ^1H NMR spectrum, e.g. two singlets of equal intensity, together equal to 3H, at 0.88 and 0.92 ppm, assignable to C-18 Me), m.p. 179–181° (Lit.[17]: m.p. 180–182°).

20α- *and* 20β-*acetoxy*-4,16-*pregnadien*-3-*one* (**13**). A soln of **12** (2.0 g, 0.0063 mole) in dry AcOH (30 ml) was allowed to stand in presence of *p*-tolune sulphonic acid (60 mg) at 15 ± 1° for 35 min. After this, water (60 ml) was added and the product taken up in ether (20 ml × 3) and worked up in the usual manner to get a product (2.1 g) showing three spots (R_f 0.20, 0.30 and 0.53, corresponding to **12**, C-20 acetate, and diacetate respectively; solvent: 50% EtOAc in light petroleum). The product was separated by chromatography on SiO_2 gel/IIb (51 cm × 1.4 cm) using light petroleum with inreasing quantities of EtOAc (2–25%) as eluant. After 2% EtOAc (50 ml × 6; 0.4764 g of diacetate) and 5% EtOAc (50 ml × 3; 0.1062 g, mixture) cuts, 10% EtOAc eluates (50 ml × 3) furnished the required C-20 acetates (*epimeric mixture*; 1.06 g), m.p. 146–148° (ether–hexane). IR ($CHCl_3$): OH 3580, 3420, 1038 cm^{-1}; OAc 1720, 1240 cm^{-1}; C=C 1660, 810 cm^{-1}. ^1H NMR: C-Me's (3H singlets at 0.85 + 0.90, and 1.03 ppm), e-CH (doublets at 1.33 and 1.38 ppm, together = 3H, each $J = 7$ Hz), OAc (3H, s, 2.03 ppm), CHOH (1H, m, 3.35–3.70 ppm), CHOAc (1H, m, 5.5 ppm), C-6H (1H, bd, 5.41 ppm), C-16H (1H, bs, 5.7 ppm).

The above product (0.4252 g, 0.0012 mole) in dry acetone (6 ml) was added to a soln of Al(OBu-t)$_3$ (0.755 g) in dry C_6H_6 (18 ml), refluxed for (12 h) and worked up as usual (5% H_2SO_4 aq., 5 ml) to furnish crude **13** as a yellow gum (0.43 g), which was purified by chromatography (SiO_2 gel/IIb, 26 cm × 1.0 cm; elution with 2–25% EtOAc in light petroleum). 5–7% EtOAc in light petroleum (25 ml × 4) gave the required product (**13**; *epimeric mixture*) 0.331 g, m.p. 127–131° (acetone–hexane). IR (CCl_4): C=C–C=O 1678, 1635 cm^{-1}: CH_3COO 1735, 1240 cm^{-1}. ^1H NMR (CCl_4): C-Me's (3H singlets at 0.84 + 0.89, and 1.20 ppm), Me–CH (doublets at 1.30 and 1.34 ppm, together = 3H, each with $J =$ 7 Hz), OAc (3H, s, 1.97 ppm). CHOAc (1H, m, 5.20–5.46 ppm), C-4H + C-16H (2H, bs, 5.59 ppm).

Z-guggulsterol (**4**). The above product (**13**; 0.331 g, 0.00093 mole) in AcOH (10 ml) and Ac_2O (1.5 ml) containing *p*-toluenesulphonic acid (20 mg), was allowed to stand at room temp (22–27°) for 72 h and then diluted with water (25 ml) and the product taken up in ether (15 ml × 3). Removal of ether furnished a yellow gum (0.33 g), which was filtered through a column of SiO_2 gel/IIb (13 cm × 1 cm). The material (semisolid, 0.32 g; single spot on TLC, R_f 0.33, 50% EtOAc in light petroleum) eluted with 2–5% EtOAc in light petroleum (150 ml) was crystallized from ether–hexane to furnish **14** (essentially C-16β-isomer), m.p. 139–143°. PMR (CCl_4): C-Me's (singlets at 0.91, 1.18 ppm), Me.CH=C(d, 1.55 ppm, $J = 7$ Hz), OAc (s, 1.97 ppm), CHOAc (m, 5.25 ppm), C–4H + C–20H (~2H, m + s, 5.57 ppm).

The above product (140 mg) was hydrolysed by refluxing (3 h) with KOH-methanolic (0.28 g KOH + 3 drops H_2O + 6 ml MeOH). Usual work-up afforded a material, which was recrystalised several times from ether–hexane to give the known[9] (16S, 17(20)Z]-16-hydroxy-4,17(20)-pregnadien-3-one (**4**), m.p. 171–173°, $[\alpha]_D + 139.7°$ (c, 1.0%), identical in all respects (m.p., mixed m.p., IR, ^1H NMR, MS) with Z-guggulsterol (Found: C, 80.12; H, 9.52. $C_{21}H_{30}O_2$ requires: C, 80.21; H, 9.62%.)

Synthesis of guggulsterol-VI (**10**)

4-*pregnene*-3,16-*dione* (**8**). Z-Guggulsterone (1.4 g, 0.0045 mole; contaminated with some 5% of E-isomer) in EtOH (50 ml) was hydrogenated at room temp. (26°) and pressure (745 nm) over 2% Pd–$SrCO_3$ (100 mg). Hydrogenation was stopped after an uptake of 1.1 molar equiv. of H_2 (~2 h) and the mixture worked up in the usual manner. The product (gum, 1.2 g; TLC, two components, R_f 0.37 and 0.50, 50% EtOAc in light petroleum) was chromatographed over SiO_2 gel/IIb (43 cm × 1.5 cm) and eluted with light petroleum containing increasing amounts (2–10%) of EtOAc: (1) 2–5% EtOAc in light petroleum, 100 ml × 5, 0.857 g, R_f 0.50, (2) 5–10% EtOAc in light petroleum, 100 ml × 5, 0.25 g, R_f 0.37; crystalline solid, m.p. 145–147°. From spectral data (^1H NMR), it was clear that fraction (2) was the desired product. Recrystallization from acetone–hexane afforded crystals, m.p. 147–148°, having spectral characteristics (also see text) consistent with structure **8**. IR (CCl_4): 1742, 1678, 1618, 1386, 1350, 1272, 1232, 1175, 948, 870 cm^{-1}. ^1H NMR: C-Me's (3H singlets at 0.76, 1.23 ppm), Me · CH_2 (3H, t, distorted t, 1.03 ppm, $J = 7$Hz), C-4H (1H, S, 5.74 ppm).

16β-*hydroxy- and* 16α-*hydroxy*-4-*pregnen*-3-*one* (**9**, **10**). The above product (0.25 g, 0.008 mole) in dry THF (10 ml) was added to a slurry of LAH (50 mg) in THF (5 ml) and the mixture stirred at room temp (28°) for 2 h. The excess LAH was destroyed by the addition of wet THF (15 ml), filtered and the filtrate freed of solvent to get a product (0.24 g) which was taken up in $CHCl_3$ (30 ml). To the $CHCl_3$ soln, active MnO_2 (3.0 g) was added and the mixture stirred at room temp for 10 h. Usual work-up furnished a product (0.24 g; TLC, at least two components, R_F 0.27 and 0.36, 50% EtOAc in light petroleum), which was chromatographed over SiO_2 gel/IIb (26 cm × 1 cm): (1) 2–7% EtOAc in light petroleum, 100 ml × 7, 0.1723 g, solid, R_F 0.36), (2) 7% EtOAc in light petroleum, 50 ml × 3, 11 mg, mixture, (3) 7% EtOAc in light petroleum, 50 ml × 3, 16 mg, solid, R_f 0.27.

Recrystallization of fraction (2) from acetone yielded crystals, m.p. 185–187°, $[\alpha]_D + 105.4°$ (c, 0.8%), recognised as **9**. IR ($CHCl_3$): OH 3420 cm^{-1}; C=O 1650 cm^{-1}; C=C 1605 cm^{-1}. ^1H NMR: C-Me's (3H singlets at 0.80, 1.19 ppm), Me–CH_2 (3H, distorted t, 0.98 ppm, $J = 7$ Hz), CHOH (1H, m, 4.29 ppm), C-4H (1H, s, 5.71 ppm). MS: *m/e* 316 (M^+, 100%). 298 (20%), 283 (10%), 274 (40%), 269 (29%), 259 (23%), 231 (26%), 175 (53), 124 (66%). (Found: C, 79.45; H, 9.94. $C_{21}H_{32}O_2$ requires: C, 79.69; H, 10.20%.)

Fraction (3) on crystallization from acetone yielded colourless needles, m.p. 197–199.5°, $[\alpha]_D + 112.2°$ (c, 0.39%) and assigned (UV, IR, ^1H NMR, MS) the structure **10**. (Found: C, 79.31; H, 10.30. $C_{21}H_{32}O_2$ requires: C, 79.69; H, 10.20%. The product was found to be identical in all respects (see text) with guggulsterol-VI.

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