Techonology Section

Pakistan J. Sci. Ind. Res., Vol. 20, Nos. 4-5, August-October 1977

STEROL COMPOSITION OF GUAR SEED OIL

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(Received December 2, 1975; revised February 19, 1977)

Abstract. GLC was used to determine sterol composition of guar meal oil. Identification of campesterol, stigmasterol, sitosterol and avenasterol was carried out by means of GLC and combined GLC-MS. The presence of cholesterol, brassicasterol, Δ^7-avenasterol and stigmast-7-enol in traces (less than 1%) was demonstrated on the basis of GLC evidence.

The plant, *Cyamposis tetragonoloba* (*C. psoralioides*), called "guar" locally belongs to the Leguminosae family. It is grown in Pakistan for its guar yielding seed coat. Industrially, guar gum is used for sizing paper and textiles, and is in great demand in the USA and Europe for these purposes.[1] It also serves as a stabilizer and filler in various food products[2], [1] and finds use in cosmetics[3] and pharmaceutical industries.[4]

The seed itself or the kernal is rich in protein, which constitutes the by-product of guar-gum industry. The kernel, when ripe, yields a dark brown, thick oil, which is reported to have medicinal value.[5] A literature survey has revealed that no work on the composition of sterols from guar oil has so far been reported. Our interest in plant sterols, therefore, prompted us to investigate the sterols of *C. tetragonoloba*.

Materials and Methods

Materials. Guar meal was purchased from the local market. Standard sterols, namely, cholesterol, brassicasterol, dihydrobrassicasterol, campesterol, stigmasterol, sitosterol, avenasterol, Δ^7-avenasterol, Δ^7-stigmasterol, 22-dehydrocholesterol, desmosterol, poriferasterol, ergosterol, fucosterol, lathosterol, 7-dehydrocholesterol, lichesterol, spinasterol, clerosterol and codisterol, were gifts from Dr. L. J. Goad, Biochemistry Department, Liverpool University.

Extraction of Oil. Guar meal was extracted with ether in a Soxhlet apparatus. After removal of the solvent a dark brown thick oil was obtained (4.3%).

Saponification. The extracted guar meal (20.9 g) was refluxed under nitrogen in alcohol (250 ml) with 50 ml. 10% KOH for 3 hr. The residue obtained from the dried ethereal extract of the saponified oil was chromatographed on basic alumina.

Column Chromatography. Petroleum ether solution of the unsaponifiable matter (1.4 g) was adsorbed on a column of basic alumina (50 g), which was developed with increasing amounts of benzene in petroleum ether. Pure benzene eluted less polar material than sterols, followed by 20 and 40% ethyl acetate in benzene, which eluted the mixture of sterols (0.524 g).

$AgNO_3$-Thin-layer Chromatography. For the separation of avenasterol for mass spectrometry, preparative silver nitrate chromatoplates (0.5 mm thickness) incorporating 10% silver nitrate in silica gel G type 60 were used. The plates were located by the Streak method and were run in 3% ether in dry chloroform free from alcohol. The following Rf values were obtained: sitosterol, campesterol and cholesterol, 0.59; stigmasterol, 0.51; Δ^7-avenasterol, 0.40; brassicasterol, 0.36; avenasterol, 0.3; and stigmast-7-enol, 0.61.

After drying the solvent the plates were sprayed with a 0.01% rhodamine 6G solution in acetone and observed under uv light (3600 A°). Areas from several plates corresponding to the marker avenasterol were cut off, scraped and extracted with ether. The residue obtained was desiccated and then was used for subsequent GLC-mass spectrometric analysis.

Gas-liquid Chromatography. GLC was performed on a Pye Unicam, Model 104 instrument equipped with a flame-ionization detector system. Coiled glass columns (1.5 meter, 6.4 mm i.d.; temp. 270°; argon flow rate =60 ml/min) packed with 3% OV-17 on Gas Chrome Q, 100-120 mesh were used. Under these conditions the retention time of sitosterol was 9 min. and 22 sec. Vidar 63 digital integrator (Autolab) was also used for determining peak areas.

GLC/MS (*AEI MS* 12). Mixtures of sterols obtained from alumina column chromatography were analyzed by GLC-MS in which a Pye 104 GLC system was coup'ed directly with spectrometer model AEI MS 12 via a Bicmann separator to remove carrier helium gas, and the total ions thus produced by the 70 eV accelarating voltage were used to monitor the affluent and detect component peaks as they emerged from the GLC column.

Mass Spectrometry. Standard mass spectrographs were produced by injecting standard sterols coated on a quartz probe directly into the mass spectrometer. The chamber potential was 70 eV.

Results and Discussion

Peaks 1-8 were identified by comparing their RRT with those of the reference sterols (Table 1). Peak areas for estimation of percentages of campesterol (14.00%), stigmasterol (15.51%), sitosterol (70.32%) and avenasterol (1%) were determined by means of Vidar 6300 digital integrator (Autolab). Δ^7-avenasterol, stigmast-7-enol, brassicasterol, and cholesterol were found in traces, *i.e.* less than 1%. The GLC chromatogram also showed one peak (IX) in traces, which could not be identified due to lack of reference standards.

TABLE 1. RELATIVE RETENTION TIME OF STANDARD AND UNKNOWN STEROLS.

Standard Sterol	Unknown	Relative rentention time	Literature value of standards
Cholesterol (Cholest-5-en-3β-ol)	Peak I	0.62	0.61
Brassicasterol ([24R]-24-methyl cholesta-5,22-dien-3β-ol)	Peak II	0.70	0.69
Campesterol ([24R]-24-methyl cholesta -5-en-3β-ol)	Peak III	0.80	0.81
Stigmasterol ([24S]-24-ethylcholesta 5,22-dien-3β-ol)	Peak IV	0.86	0.88
Sitosterol ([24R]-24-ethylcholesta -5-en-3β-ol)	Peak V	1.00	1.00
Avenasterol ([24Z]-24 ethylidene cholesta-5-en-3β-ol)	Peak VI	1.10	1.12
Δ^7-Stigmasterol ([24R]-24-ethylcholesta-7-en-3β-ol	Peak VII	1.16	1.18
Δ^7-avenasterol ([24Z]-24-etylidene cholesta-7-en-3β-ol)	Peak VIII	1.31	1.32
Unknown	Peak IX	1.46	—

The reference standards showed RRTs, which were exactly the same as recorded for the sterols from guar meal oil.

Retention time of sitosterol (retention time=9 min. and 22 sec.) was taken as 1.00. See text for operating conditions of GLC.

Campesterol, stigmasterol, sitosterol and avenasterol were also identified by comparing their fragmentation pattern with that of the reference standards in GLC-MS.

The fragmentation pattern of the principal peaks of these sterols is given in Table 2, which compares favourably with the m/e peaks of standard sterols.

The sterol with RRT: 1.1 (peak VI) gave the molecular ion at m/e 412 with other characteristic ions at m/e 397 ($M^+—CH_3$) and 397 ($M^+—CH_3+H_2O$). The presence of ethylidence group at C-24 is indicated by a peak at m/e 314, which arises from the loss of part of side chain ($—C_7H_{14}$) by a McLafferty rearrangement and is typical of sterols with a Δ^{24} (^{28}) bond[6-9]. Other ion most characteristic of $\Delta^{5,24}$ (^{28}), sterols is $M^+—$ (part of side chain$+H_2O$) which appears at m/s 296.

The sterol in question was, therefore, identified as avenasterol.

GLC-MS for peak number III (Table I) shows a molecular ion at m/e 400. The m/e 255 fragment indicates unsaturation at C-5 and C-6. Another fragmentation ion resulting from the removal of side chain, which occurs at m/e 273, is the same as found in cholesterol. This indicates that the difference lies in the side chain. Since the molecular weight differrence between peak III and cholesterol is 14, it is implied that a methyl group is involved in the case of peak III. In plants, whenever any group is found in the side chain of any sterol, it is invariably associated with C-24 atom of the side chain. Further, it is also known, that in higher plants such groups have a 24α (24R) configuratian except for the 25-methylene sterols.[10] From this discussion and also from the fact that the m/e peaks of standard campesterol were essentially similar, peak III sterol was identified as campesterol.

A similar discussion may be advanced for peak V, where instead of a methyl group an ethyl group seems to be involved. Comparing its fragmentation pattern with that of standard sitosterol, this peak may be identified as sitosterol.

Δ^{22}-sterols are characterized by the loss of 43 mass units (terminal *iso*-propyl group). The GLC-MS for peak IV shows a molecular ion at m/e 412 and other characteristic peaks at m/e 369 ($M^+—43$) and at m/e

351 (M^+-43+H_2O). This indicates that the compound has a Δ^{22} double bond. The principal peak fragmentation as given in Table 2 compares well with the fragmentation obtained from standard stigmasterol. This peak was, therefore, identified as stigmasterol.

The evidence presented in this paper shows that the sterols of guar meal oil are a complex mixture in which sitosterol predominates. Although cholesterol is regarded as an animal sterol its presence in this oil has been demonstrated on the basis of the GLC findings.

Acknowledgment. Thanks are due to Mr. M. Aslam, Director, Karachi Laboratorsies for his encouragement in this work.

TABLE 2. PRINCIPAL FRAGMENTATION OF PEAKS III, IV, V, AND VI.

m/e	Peak III	Peak IV	Peak V	Peak VI
M^+ Molecular ion	400 (100)	412 (100)	414 (100)	412 (15)
M^- $-CH_3$	385 (31)	397 (12)	399 (27)	397 (2)
M^+ $-H_2O$	382 (54)	394 (8)	396 (36)	394 (1)
M^+ $-CH_3+H_2O$	367 (29)	379 (25)	381 (24)	379 (1)
M^+ $-H_2O+67$ (C_5H_7)	315 (28)	—	329 (32)	—
M^+ H_2O+93 (C_7H_9)	289 (56)	—	303 (52)	—
M^+ $-H_2O+93+1\times H$	—	300 (45)	—	—
M^+ $-H_2O+121$ (C_9H_{13})	261 (12)	273 (21)	275 (12)	—
M^+ -43 (C_{25}—C_{27})	—	369 (23)	—	—
M^+ $-43+H_2O$	—	351 (29)	—	—
M^+ $-C_{23}$—$C_{27}+1\times H$	—	314 (10)	—	314 (—)
M^+ $-C_{23}$—$C_{27}+1\times H+CH_3$	—	—	—	299 (—)
M^+ $-C_{23}$—C_{27} (C_7H_{14})$+H_2O$	—	—	—	296 (—)
M^+ -27 (C_{16}—C_{17})	—	—	—	385 (—)
M^+ —Side chain	273 (31)	—	273 (36)	—
M^+ —Side chain $+2\times H$	—	271 (—)	—	271 (—)
M^+ —Side chain $+H_2O$	255 (—)	255 (—)	255 (42)	255 (—)
M^+ —Side chain $+42$	231 (—)	231 (—)	231 (40)	—
M^+ —Side chain $+43$	—	—	—	231 (—)
M^+ —Side chain $+42+H_2O$	213 (—)	213 (—)	213 (60)	—
M^+ —Side chain $+43+H_2O$	—	—	—	213 (—)
M^+ —Side chain $+27$ (C_{16}—C_{17})	—	—	—	246 (—)

Figures in parenthesis indicate the relative abundance for ions measured in the range m/e 210 to the molecular ion only. A dash indicates that the indicated ion or the relative abundance was either not recorded or was not observable.

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