

## INVESTIGATION OF EPHEDRA GREASE FROM MARKER ALKALOIDS, QUETTA

A. WADOOD QURESHI AND A.M. AHSAN

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received November 17, 1965)

Ginnol or 10-nonacosanol, nonacosane, n-octacosanol and  $\beta$ -sitosterol have been isolated and identified from the grease left as a by-product in the manufacture of the alkaloid, ephedrine, by Marker Alkaloids of Quetta.

### Introduction

During the manufacture of ephedrine, the benzene/petroleum ether extractive of the alkaline plant material leaves after separation of the bases with hydrochloric acid a dark green, almost black, grease, in appreciable quantities as a by-product.

The grease has been under examination in these Laboratories for some time now in order to evaluate its industrial possibilities. In the course of previous investigations by N.U. Siddiqui and G. Hahn<sup>1</sup> three substances, *viz.*, a secondary alcohol, a hydrocarbon and a sterol have been reported.

The present authors have identified these materials as ginnol or 10-nonacosanol, nonacosane, and  $\beta$ -sitosterol respectively as detailed below. In addition they have isolated another alcohol, n-octacosanol.

The industrial uses of the grease will be communicated separately.

The general outline of the method of working employed to isolate and identify the products mentioned is that of Siddiqui and Hahn,<sup>1</sup> but in this work milder procedures have been usually followed to advantage. For example, the grease was saponified with 0.5 N alcoholic potash instead of ca. 4.5N of the previous authors and the time of treatment with the alkali was 6 hours instead of 16 used by them. After extracting out the unsaponifiable matter with ether, the former gave crystals from alcohol, m.p. 67°C (unsharp). The latter were chromatographed on alumina, to yield ginnol, nonacosane and n-octacosanol.

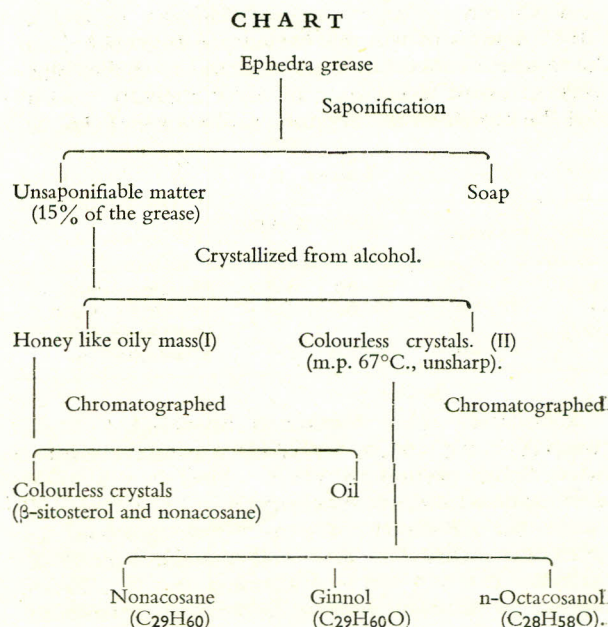
Ginnol has been identified by the preparation of its acetate and hydrogen phthalic ester.<sup>2</sup> The benzoyl derivative was also prepared in order to compare it with the benzoate of celidoniol,<sup>3</sup> which latter has been identified as 10-nonacosanol,<sup>4</sup> and the melting point quoted by the author compares with that of our benzoate.

However, the identity of the two substances will be communicated later.

In the case of nonacosane, the methods of purification employed by Siddiqui and Hahn<sup>1</sup> (crystallisation from acetone) did not yield the desired results and thus various other solvents were tried without much effect. Ultimately recourse to chromatography gave us the pure sample, whose identity was established through its infrared spectrum and the refractive index above its melting point.

In the case of n-octacosanol, only one derivative, *i.e.*, the acetate<sup>5</sup> was prepared as no other has so far been reported in the literature.

The matter remaining in the mother liquor was an oily mass(I) which was chromatographed over alumina, when further quantities of nonacosane and  $\beta$ -sitosterol were obtained. The procedure of separation is summarized in the chart below:



The oil left after separation of  $\beta$ -sitosterol is under investigation.



### Experimental

All melting points are uncorrected and unless otherwise mentioned they are presumed to be taken in the ordinary way with a melting point capillary.

All analyses were done by Mr. Alfred Bernhardt, Mikroanalytisches Laboratorium im Max-Planck-Institut für Kohlenforschung, Mulheim (Ruhr), West Germany.

All the experiments in chromatography have been done with alumina Brockmann, grade II to III (E. Merck).

The grease (500 g.) was saponified according to the general method of the British Pharmacopeia<sup>6</sup> using 0.5N alcoholic potassium hydroxide (3 l.). After refluxing for 6 hours the solvent was removed *in vacuo* from the brown solution, and the concentrate diluted with water (6 l.). On extraction with ether and working up the extract in the usual way the unsaponifiable matter was obtained as a yellow oily mass (75 g.; 15% of the grease). It gave colourless crystals from alcohol, m.p. 67°C (unsharp) (32.7 g.; 7%). The mother liquor on evaporation left a honey like oily mass (I, see Chart).

The crystalline substance isolated above, m.p. 67°C (14 g.) (II, see Chart) was dissolved in petroleum ether (70 ml.) and chromatographed on a column (50 × 3.5 cm.) of alumina (400 g.). Fifty fractions of 100 ml. each were collected, and the residues from them after removal of the solvent were grouped according to their melting points and the nature of the crystals, as shown in Table I.

TABLE I.

Group	Fractions	M.P. °C.	Weight g.	Eluting solvent
A	1—21	63—64	1.8	Petroleum ether.
B	22—36	79—80	9.0	Petroleum ether: chloroform (1:1).
C	37—50	71—72	2.5	Chloroform.

*Nonacosane*.—The combined material from group A (1.8 g.), m.p. 63–64°C, was recrystallised twice from petroleum ether. However, as the melting point did not rise but showed an increased range, the substance (900 ml.) was dissolved in petroleum ether (10 ml.) and passed through a column (22 × 2.5 cm.) of alumina (60 g.). Fractions of 20 ml. each were collected with petroleum ether as the eluent. The combined residues obtained after removal of the solvent from the different fractions (898 mg.) were again recrystallised from petroleum ether to give a m.p. of 63–65°C,

which though higher exhibited an increased range. The substance (898 mg.) was therefore dissolved in petroleum ether (10 ml.) again and passed through alumina a third time, when the melting point rose to 64.5–65°C. Two further crystallisations from petroleum ether afforded colourless needle-shaped crystals m.p. same (lit., 64–65°C.)<sup>7</sup> (Found: C, 84.59; H, 14.96%; mol. wt. 408; Calc. for C<sub>29</sub>H<sub>60</sub>: C, 85.20; H, 14.80%; mol. wt. 408.8).

It showed peaks at 2925, 2875, 1460, 1385, 731, and 721 cm<sup>-1</sup>. in the infrared spectrum (KBr). There was neither bromine absorption nor decolourisation with permanganate solution; hence the hydrocarbon was saturated and this was also indicated by its I.R. spectrum. Its refractive index was determined  $n_D^{70}$  1.43456, (Lit.,  $n_D^{70}$  1.4340,<sup>8</sup>  $n_D^{65}$  1.43640,  $n_D^{80}$  1.43061).<sup>9</sup>

*Ginnol*.—The residue from mixed group B fractions (Table I), m.p., 79–80°C, was recrystallised thrice from benzene to give the analytical sample, m.p., 81.5–82°C, slightly dextrorotatory in chloroform solution. (lit., 81.5–82°C.; slightly dextrorotatory in chloroform solution).<sup>2</sup> (Found: C, 82.06; H, 13.94; O, 3.99%; mol. wt. 429; Calc. for C<sub>29</sub>H<sub>60</sub>O: C, 81.99; H, 14.24; O, 3.77%; mol. wt. 424.8).

Its infrared spectrum (KBr) indicated the presence of hydroxyl group or groups and exhibited peaks at 3325, 1375, 1345, 1140, 1090, 860 and 721 cm<sup>-1</sup>. It gave negative Liebermann-Burchard test and was found saturated as it did not absorb bromine and did not decolourise permanganate solution.

The *acetate* was prepared by refluxing the substance (300 mg.) with dry pyridine (6 ml.) and acetic anhydride (6 ml.) for one hour. It was poured into water and the precipitate of acetate (335 mg.), m.p. 43–44°C, was sucked off. On recrystallisation thrice from acetone colourless crystals were obtained with the constant m.p. 44°C. (lit., 44–46°C,<sup>2</sup> (Found: C, 79.78; H, 13.44; O, 7.04%; mol. wt. 450; Calc. for C<sub>31</sub>H<sub>62</sub>O<sub>2</sub>: C, 79.76; H, 13.39; O, 6.86%; mol. wt. 466.81).

Its infrared spectrum (KBr) showed bands at 1740, 1370, 1240 and 1050 cm<sup>-1</sup>.

The *hydrogen phthalic ester* was made by heating ginnol (848 mg.) and freshly prepared phthalic anhydride (326 mg.) at the temperature of 110–120°C, for ten hours the method followed being the general one of Pickard and Kenyon.<sup>10</sup> The subsequent treatment of the product is,



however, different and consists simply in taking it up in chloroform and washing repeatedly with water. The organic layer on drying over calcium chloride and removal of the solvent gave a colourless syrupy liquid which slowly and completely crystallised in beads (975 mg.; 86%), m.p. 53-54°C. Two recrystallisations from petroleum ether (b.p. 60-80°C.) gave clusters of colourless needles (467 mg.) m.p. 54.5-55°C. (lit., m.p. 54.5-54.7°C.).<sup>2</sup>

In a 2% chloroform solution the substance was found to be slightly dextrorotatory, but the rotation was not measureable (lit.,<sup>2</sup> slightly dextrorotatory but not measureable). It is readily soluble in chloroform, ethyl acetate and sparingly soluble in petroleum ether, ethanol and methanol.

*Ginnol Benzoate*.—The substance (300 mg.), benzoyl chloride (2 ml.) and pyridine (6 ml.) were mixed and refluxed for two hours. On cooling, the solution was poured into cold water and extracted with chloroform. The organic layer was extracted with hydrochloric acid solution (1 N), sodium bicarbonate solution (5%) and finally washed with water. The crude benzoate, m.p. 34-35°C, was recrystallised thrice from ethanol to give the colourless analytical sample, m.p. 35-36°C. (Found: C, 81.81; H, 12.32; O, 6.07%; mol. wt. 551; C<sub>36</sub>H<sub>64</sub>O<sub>2</sub> requires; C, 81.75; H, 12.20; O, 6.05%; mol. wt. 528.87).

It furnished peaks in the infrared spectrum (KBr) at 1720, 1265 and 1110 cm<sup>-1</sup>.

*n-Octacosanol*.—The fractions (37-50) of the group C (2.5 g.) m.p. 71-72°C, were combined and recrystallised thrice from benzene. The melting point reached upto 83.5-84°C. The alcohol (43 mg.) was then chromatographed over alumina (10 g.) on a column (12 × 1.5 cm.). Eight fractions of 20 ml. were collected with a mixture of petroleum ether and benzene (1:1) as the eluent (Table 2):

TABLE 2.

Group	Fractions	Amount mg.	M.P. (Kofler). °C.
1.	1-7	38.5	81-83
2.	8	4.0	70-75

The first seven fractions were combined and recrystallised twice from acetone/benzene, m.p. 84-85°C. (Kofler) (lit., m.p. 84°C).<sup>5</sup> (Found: C, 81.49; H, 14.07; O, 4.09%; Calc. for C<sub>28</sub>H<sub>58</sub>O: C, 81.87; H, 14.23; O, 3.90%).

The infrared spectrum (KBr) differed from that of ginnol and showed peaks at 3325, 2925, 1460, 1060, 730 and 721 cm<sup>-1</sup>. There was neither bromine absorption nor decolourisation of permanganate solution; hence the alcohol was saturated.

The *acetate* was prepared by refluxing the alcohol (92 mg.) with acetic anhydride (2 ml.) and pyridine (2 ml.) for one hour instead of heating with the anhydride and fused sodium acetate the method of Chibnall and co-workers.<sup>11</sup> After cooling the solution was poured into water and the precipitate sucked off. On recrystallisation thrice from the mixture of ethanol and acetone colourless crystals, m.p. 68.5-69°C (Kofler) were obtained. (lit., m.p. 68.2°C).<sup>5</sup> (Found: C, 79.32; H, 12.94%; Calc. for C<sub>30</sub>H<sub>60</sub>O<sub>2</sub>: C, 79.57; H, 13.36%).

*β-Sitosterol*.—The honey like oily mass (22.4 g.; I, see Chart) obtained on removal of the solvent after separation of ginnol, nonacosane and octacosanol was submitted to chromatography on a column (50 × 6 cm.) of alumina (1 kg.). The mixtures petroleum ether/benzene (1:1), petroleum ether/chloroform (1:1) and methanol/chloroform (1:3) were used as eluents and finally pure methanol. Fractions of 100 ml. were collected throughout. In all 44 fractions were collected which were divided into two groups on physical examination (Table 3):

TABLE 3.

Group	Fractions	Weight g.	Remarks
I	1-33	5	Yellow oily semi-solid.
II	34-44	17	Brown oily semi-solid.

Fractions of group I were combined and crystallised from alcohol giving colourless crystals, m.p. 63-64°C., which on repeated recrystallisation from petroleum ether yielded further quantities of nonacosane.

Fractions of group II when combined and crystallised from ethanol gave colourless needle-shaped crystals, (0.9 g.) m.p. 137-138°C, (lit., 136-137°C<sup>12</sup>) which on recrystallisation from ethyl alcohol did not show any increase in the melting point.

The substance gave positive Salkowski and Liebermann-Burchard tests for sterol. It decolourised the permanganate solution and bromine



in chloroform was absorbed thus showing that it was unsaturated. Its infrared spectrum (KBr) was found identical with an authentic sample of  $\beta$ -sitosterol with which the mixed m.p. was undepressed.

The acetate of the sample prepared in the usual way, by heating with acetic anhydride and pyridine, m.p., 127-128°C (lit., 126-127°C).<sup>12</sup>

**Acknowledgement.**—The authors acknowledge with thanks the interest of Dr. Salimuzzaman Siddiqui, F.R.S., Chairman, Pakistan Council of Scientific and Industrial Research in the progress of the work. Thanks are also due to Marker Alkaloids of Quetta for the ready supply of grease, the raw material of the work, and to Messrs. Khushal Khan and Mohammad Eshaque for the infrared spectra.

#### References

1. Naseem Uddin Siddiqui and G. Hahn, Pakistan J. Sci. Ind. Res., **2**, 245 (1959).

2. Chibnall *et al.*, Biochem. J., **25**, 2108 (1931).
3. E. Seoane, Anales Real Soc. Espana Fizikai Quimica (Madrid), Ser. B, 58 No. 1, 69-74 (1962).
4. *Idem.*, *ibid.*, p. 75.
5. Chibnall *et al.*, Biochem. J., **28**, 2197 (1934).
6. British Pharmacopoeia, 875 (1958).
7. Beilsteins Handbuch der Organischen Chemie, vol. I, 4th ed., 2nd supplement, p. 144.
8. *Ibid.*, 3rd Supplement, p. 584.
9. Glud, Ber., **52**, 1040 (1919).
10. R.H. Pickard and J. Kenyon, J. Chem. Soc., **103**, 1937 (1913).
11. Chibnall *et al.*, Biochem. J., **27**, 1889 (1933).
12. G. Hahn, A.M. Ahsan and A.W. Qureshi, Pakistan J. Sci. Ind. Res., **7**, 219 (1964).