323. *The Delphinine Alkaloids. Part I.*

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From the seeds of *Delphinium staphisagria*, in addition to the known alkaloids delphinine and staphisine, a new crystalline base, $C_{20}H_{29}O_5N$, has been isolated and its ultra-violet absorption spectrum measured. On dehydrogenation with selenium, a phenanthrene-type hydrocarbon, $C_{18}H_{16}$, has been obtained.

The unsaponifiable non-basic fraction from the same seeds contains β-sitosterol.

Since the beginning of the nineteenth century many workers have examined the basic content of *Delphinium staphisagria* L. seeds (commonly known as stavesacre). In addition to delphinine ($C_{33}H_{45}O_9N$) and the recently isolated staphisine ($C_{22}H_{31}ON$ or $C_{42}H_{60}ON_2$), mention is made in the older literature (Marquis, *Arch. exp. Path. Pharmakol.*, 1877, **7**, 55—80; *Pharm. Z. Russland*, 1877, **16**, 449, 481, 513; Kara-stojanow, *Pharm. Z. Russland*, 1890, **29**, 628, 641, 657, 673, 689, 705, 721; abstracted in *Jahresber.*, 1877, 894—897, and *J.*, 1891, **60**, 842; Ahrens, *Ber.*, 1899, **32**, 1581) of the occurrence of other alkaloids, but none of these has been characterised.

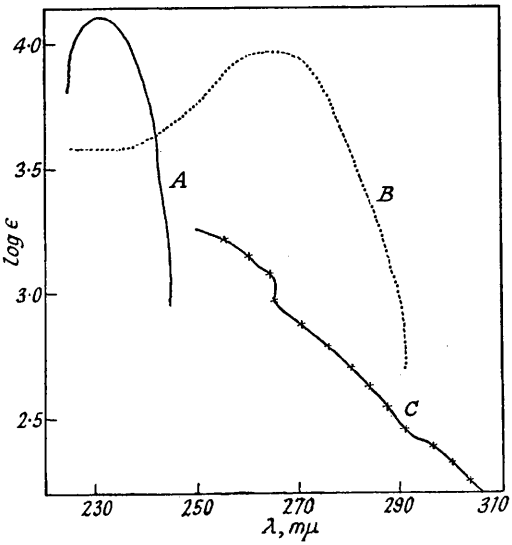
Jacobs and Craig (*J. Biol. Chem.*, 1941, **141**, 67) recorded the presence of a very small quantity of a sparingly soluble alkaloid, m. p. 300° (decomp.), in the mother-liquors from delphinine and staphisine.

We have found that after delphinine and staphisine have been extracted from the powdered seeds with light petroleum, chloroform removes further basic material from which a colourless crystalline base, $C_{20}H_{29}O_5N$, m. p. 286—290° (decomp.), can be obtained. This forms a crystalline oxalate, but only an amorphous hydrochloride and methiodide. On catalytic hydrogenation a dihydro-derivative is obtained.

The ultra-violet absorption spectra of delphinine, staphisine, and the new alkaloid have been compared. The values for the maxima of the first two alkaloids are in good agreement with those recorded (Jacobs and Craig, *loc. cit.*; Brustier and Vignes, *Bull. Soc. chim.* 1945, **12**, 37), but the nature of the curve for the new alkaloid is entirely different as shown in Fig. 1.

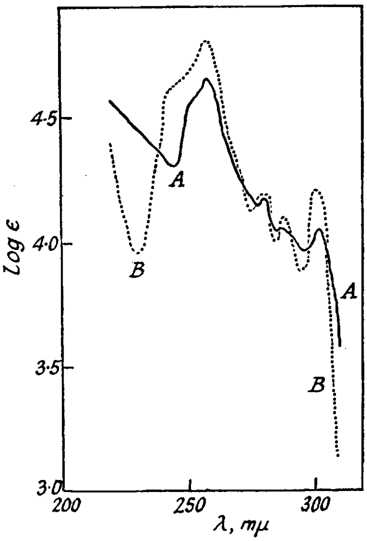
On dehydrogenation with selenium at 330—350° in nitrogen, the new base gives a mixture of hydrocarbons, of which only one has so far been isolated in a pure form. It melts at 51—52° and forms a picrate, and analyses indicate the formula $C_{18}H_{16}$. Its ultra-violet absorption spectrum (Fig. 2) clearly indicates that it is a phenanthrene derivative.

FIG. 1.



A, Delphinine; B, staphisine; C, new alkaloid.

FIG. 2.



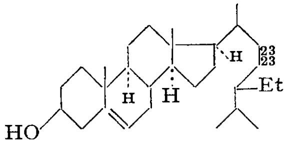
A, Hydrocarbon, $C_{18}H_{16}$, *in ethanol; B,* 1 : 2-cyclo*pentenophenanthrene.*

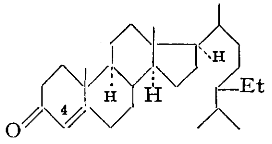
The nature of the curves, the positions and the intensities of the principal absorption maxima of various phenanthrene compounds lead to the conclusion that our hydrocarbon is either a *cyclo*penteno- or an alkyl-phenanthrene (Mayneord and Roe, *Proc. Roy. Soc.*, 1935, *A*, **152**, 317; Heilbronner, Daniker, and Plattner, *Helv. Chim. Acta*, 1949, **32**, 1723; Jacobs and Craig, *loc. cit.*; the *cyclo*penteno- and alkyl portions of these compounds do not produce any marked differences in the ultra-violet absorption, and it is not possible to decide on this evidence alone to which group our hydrocarbon belongs.

In 1927, Markwood (*J. Amer. Pharm. Assoc.*, 1927, **16**, 928), when reporting the insecticidal activity of the oil present in the seed of *D. staphisagria*, observed that the oil contains 2·92% of unsaponifiable matter. The latter has been investigated in this laboratory with the object of isolating pure compounds whose structure might have, directly or indirectly, some relation to the accompanying alkaloids. On chromatography the unsaponifiable portion yielded a number of substances, including β-sitosterol (22 : 23-dihydrostigmasterol) (I). The identity of this was confirmed by its conversion into β-sitost-4-enone (II), which was characterised as its oxime and dinitrophenylhydrazone.

By selenium dehydrogenation of staphisine Jacobs and Huebner obtained pimanthrene and retene, and in this laboratory the new alkaloid has also given a hydrocarbon containing a phenanthrene nucleus. β-Sitosterol is the only accompanying phenanthrene derivative so far isolated in a pure condition from *D. staphisagria* seeds and may therefore be structurally related to the *Delphinium* alkaloids. Further work on the constitution of delphinine and other alkaloids of *D. staphisagria* seed and on the elucidation of the relation to the aconitine bases is in progress.

(I)





(II)

EXPERIMENTAL

Extraction of the Alkaloid.—The residue, after extraction of delphinine and staphisine by shaking the ground seeds with light petroleum (b. p. 40—60°) for 24 hours, was extracted (Soxhlet) with chloroform, and the dark oil thus obtained was extracted with 2% sulphuric acid. The combined acid extracts were washed with ether and light petroleum, basified (sodium hydroxide), and extracted with ether. On removal of the ether, light yellow, amorphous, basic material was obtained. This was shaken with 3% tartaric acid solution, then centrifuged, and the clear acid solution was basified and extracted with ether. The colourless residue from this extract was chromatographed in benzene on alumina. Elution with chloroform and methanol yielded a further very small quantity of delphinine and staphisine and a crystalline *base* (0·2—0·3 g. per kg. of commercial seeds), m. p. 270—280° (decomp.). Recrystallisation from methanol–ether raised the m. p. to 286—290° (decomp.) (slight colouration from 260°), $[\alpha]_D$ −19·23° in EtOH [Found: C, 66·4, 66·3; H, 8·15, 8·2; N, 3·9%; *M* (cryoscopic in bromoform), 368. $C_{20}H_{29}O_5N$ requires C, 66·1; H, 8·0; N, 3·9%; *M*, 363. Jacobs and Craig found for their base: C, 80·7; H, 8·8; N, 4·4%].

Salts.—To the base (12 mg.), dissolved in the minimum quantity of ethanol, were added a few drops of saturated alcoholic oxalic acid. The white precipitate was collected, washed with ether, and crystallised from ethanol, giving aggregated prisms of the *hydrogen oxalate*, m. p. 300—305° (decomp.) (Found: C, 58·5; H, 6·6. $C_{20}H_{29}O_5N,C_2H_2O_4$ requires C, 58·3; H, 6·8%).

An attempt to prepare a hydrochloride gave an amorphous precipitate, m. p. 290—295° (decomp.).

Hydrogenation.—The base (4·65 mg.) in methyl alcohol (10 ml.) containing a drop of acetic acid was hydrogenated with platinic oxide (15 mg.), hydrogen (0·3 ml.) being quickly absorbed. The reaction mixture was filtered, the solvent removed, and the *dihydro*-compound crystallised from methanol–ether, giving stout prisms, m. p. 235—238° (Found: C, 66·2; H, 8·8. $C_{20}H_{31}O_5N$ requires C, 65·75; H, 8·5%).

Dehydrogenation.—The alkaloid (1·1 g.) and selenium (4 g.) were heated in nitrogen to 345° for 6 hours. A trace of volatile material was trapped in an ice–salt mixture. The reaction mixture was extracted with hot benzene, the extract treated with 10% hydrochloric acid and then 5% sodium hydroxide solution, washed with water, and dried (Na_2SO_4), and the solvent removed. The oil was freed from selenium by distillation over sodium. The resinous product was chromatographed in benzene on alumina, and eluted with ether and chloroform. One fraction (20 mg.) distilled at 170—180° (bath-temp.)/0·2 mm., and when kept in a refrigerator for 2 days yielded a semicrystalline *hydrocarbon*. Crystallised from ethanol, this had m. p. 51—52° and showed a light blue fluorescence (Found: C, 93·5; H, 7·05. $C_{18}H_{16}$ requires C, 93·1; H, 6·9%). Its *picrate*, formed in ether and recrystallised from ethanol, had m. p. 145—147°.

Isolation of β-Sitosterol.—The alkaloid-free seed oil (225 g.) and methanolic potassium hydroxide (1 l.; 2N) were heated under reflux overnight. Part of the methanol was distilled off and the residue poured into water and extracted with ether. After removal of the ether, the residue was again heated with methanolic potassium hydroxide (125 ml.; 2N) for 2 hours and the mixture treated as before. The ethereal extract was washed twice with water, dried (Na_2SO_4), and distilled, yielding a semi-solid material (6·9 g., 3·1%). This was chromatographed in benzene–light petroleum (b. p. 40—60°) (1 : 1) on alumina and eluted with ether and chloroform. In the initial fraction a low melting solid (4·5 g.) with a terpene-like odour was obtained, but this was followed by the steroid (250 mg.) which gave colourless, shining plates (from methanol), m. p. 137—138°, $[\alpha]_D$ −31·90° (Found: C, 84·0; H, 12·4. Calc. for $C_{29}H_{50}O$: C, 84·1; H, 12·1%). It gave the Liebermann, Liebermann–Burchard, Salkowski, Rosenheim, and Tschugajeff colour tests.

The acetate, prepared in acetic anhydride–pyridine and crystallised from methanol, formed colourless prisms, m. p. 127°, not depressed by admixture with authentic material (Found: C, 81·2; H, 11·05. Calc. for $C_{29}H_{49}$·OAc: C, 81·6; H, 11·4%).

The benzoate formed colourless prisms (from ether–methanol), m. p. 141°, not depressed by admixture with an authentic specimen (Found: C, 83·2; H, 10·9. Calc. for $C_{29}H_{49}O·CO·C_6H_5$: C, 83·4; H, 10·5%).

The digitonide (30·8 mg.) was obtained from the sterol (7·8 mg.).

The sterol (300 mg.) in acetone (6 ml.) was refluxed with aluminium *tert.*-butoxide (600 mg.) in benzene for 24 hours. The solution was washed with 2N-sulphuric acid and then water. After drying (Na_2SO_4) and removal of mesityl oxide, the product was chromatographed in light petroleum on alumina and eluted with benzene and ether. β-Sitost-4-enone (100 mg.) was obtained, having m. p. 87—88°, $[\alpha]_D$ +85·50° in ethanol (Found: C, 84·3; H, 11·6. Calc. for $C_{29}H_{48}O$: C, 84·5; H, 11·6%). Barton and Jones (*J.*, 1943, 599) have recorded m. p. 88° and $[\alpha]_D$ +85·88°.

The 2 : 4-dinitrophenylhydrazone was obtained as red prisms, m. p. 251—253° (decomp.) [Jones, Wilkinson, and Kerlogue, *J.*, 1942, 391, report m. p. 253° (decomp.)], and the oxime as colourless prisms, m. p. 177° (*idem, ibid.*, record m. p. 175—175·5°) (Found: C, 80·95; H, 11·3. Calc. for $C_{29}H_{49}ON$: C, 81·5; H, 11·5%).

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