

116. *Nyctanthic Acid, a Constituent of Nyctanthes arbor-tristis* *Linn.*

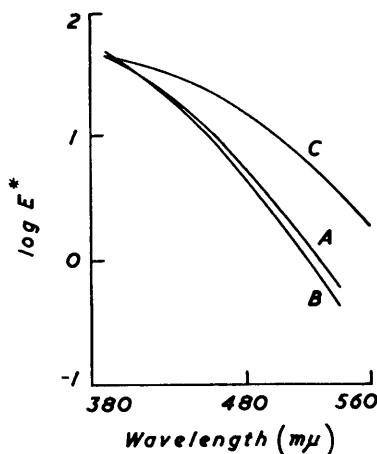
By J. H. TURNBULL, SHYAM KISHORE VASISTHA, W. WILSON,
and R. WOODGER.

β -Sitosterol and nyctanthic acid have been isolated from *Nyctanthes arbor-tristis* seeds. Nyctanthic acid, probably $C_{30}H_{48}O_2$, appears to be a novel type of tetracyclic triterpenoid acid.

THE shrub *Nyctanthes arbor-tristis* Linn. (Harsinghar, Parijataka) is widely cultivated and grows wild throughout India,¹ and various extracts of the plant have been credited with medicinal properties. Several years ago, one of us² investigated the seed oil and identified a number of glycerides, together with a sterol, m. p. 130° , $[\alpha]_D -22^\circ$, and a substance, m. p. 222° , $[\alpha]_D +91^\circ$, formulated as $C_{27}H_{44}O_2$ and named "nycosterol."

We have re-investigated the steroid components. The seed oil, obtained in about 15% yield from the dried kernels, slowly deposited crystals of "nycosterol," and the non-saponifiable residue from the remaining oil afforded a sterol having properties in excellent agreement with those reported³ for β -sitosterol.

Absorption of:
(A) *Nyctanthinol* (likewise *dihydronyctanthinol*).
(B) *3-Acetoxyergosta-7:22-diene*.
(C) *Lanost-8-enol*.
 E^* is as defined by Heilbronner.⁷



Analysis of "nycosterol" and its derivatives indicated the probable molecular formula $C_{30}H_{48}O_2$, although close homologues such as $C_{29}H_{46}O_2$ or $C_{31}H_{50}O_2$ cannot be excluded. "Nycosterol" is a carboxylic acid, as it forms an amide and a highly crystalline methyl ester, but it is insoluble in alkalis, insoluble salts being formed; the acidic nature of the substance is therefore not immediately evident. The substance is accordingly renamed nyctanthic acid. The molecular formula $C_{30}H_{48}O_2$ (or $C_{29}H_{46}O_2$) of the acid suggests a tetracyclic triterpenoid structure with two double bonds; catalytic microhydrogenation established the presence of one easily reducible double bond, giving dihydronyctanthic acid. There is little change in optical rotation on reduction of the reactive double bond; this is a known feature of the tetracyclic triterpenoids.^{4, 5}

Reduction of methyl nyctanthate by lithium aluminium hydride yielded the primary

¹ Hooker, "The Flora of British India," Reeve, Ashford, 1882, Vol. 3, p. 603; Kurz, "Forest Flora of British Burma," Govt. Printing Office, Calcutta, 1877, Vol. 2, p. 155; Burkill, "A Dictionary of the Economic Products of the Malay Peninsula," Crown Agents for the Colonies, London, 1935, Vol. 2, p. 1564.

² Vasistha, *J. Benares Hindu Univ.*, 1938, 2, 343; D.Sc. Thesis, Benares, 1940.

³ Josephy and Radt, "Encyclopedia of Organic Chemistry," Elsevier, Amsterdam, 1940, Vol. 14, p. 90; 1954, Suppl. Vol. 14, p. 1808 S.

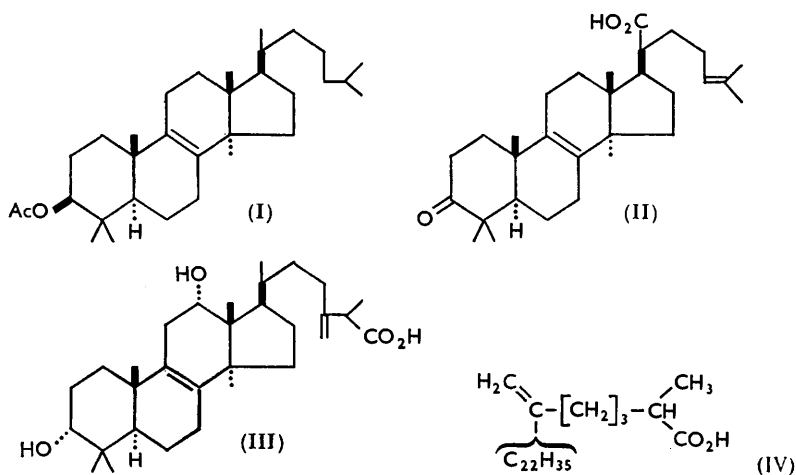
⁴ Barton and Jones, *J.*, 1944, 659.

⁵ Jones and Woods, *J.*, 1953, 464.

alcohol, nyctanthinol. The latter (and therefore nyctanthic acid also) possessed a vinylidene (>C=CH_2) group, as shown by infrared absorption bands at 1634 and 890 cm^{-1} ; dihydrynyctanthinol does not show these bands. Nyctanthic acid rapidly consumed 1 mol. of bromine, but only 0.3 equiv. of monoperphthalic acid reacted in 16 hr. The vinylidene group is most readily accommodated in the side-chain of a tetracyclic triterpenoid structure: it has been noted⁶ that certain steroid side-chain double bonds react only slowly with peracids. Although dihydrynyctanthic acid did not react with either bromine or monoperphthalic acid, a second double bond was believed to be present. Thus, dihydrynyctanthinol gave a colour with tetranitromethane, the absorption curve (Fig.) determined by Heilbronner's method⁷ being consistent with the presence of a trisubstituted double bond. The nature of the ultraviolet light absorption near 200 $\text{m}\mu$ of dihydrynyctanthinol suggested the presence of a trisubstituted double bond (cf. Bladon, Henbest, and Wood⁸). Dihydrynyctanthinol had a moderately weak infrared absorption band at 809–810 cm^{-1} , further consistent with the presence of a trisubstituted double bond.

Nyctanthic acid gives a resinous adduct with hydrogen chloride and is regenerated therefrom by treatment with alkali. The side-chain vinylidene group is doubtless implicated in these changes (cf. *cyclolaudenol*⁹).

Nyctanthic and dihydrynyctanthic acid are substantially unaffected by boiling acetic acid containing a little sulphuric acid. It is inferred that *cyclopropane* rings (cf. *cycloartenol*^{10a, b} and *cyclolaudenol*⁹) are absent. On the assumption that the acid has a conventional type of skeleton (e.g., of the lanostane type), the inert double bond probably



occupies the 7:8- or 9:11-position. In the lanostane series 7:8-double bonds resist hydrogenation^{11a} and, in contrast to the case in the euphol series,^{11b} are not appreciably rearranged by mineral acids. Thus Holker *et al.*¹² recovered 3-acetoxy-euburic-7-enoic acid unchanged after treatment with mineral acid, while the stable $\Delta^7:8$ -isomer results from partial rearrangement of lanost-8-enyl acetate (I) by hydrogen chloride.^{11, 13} 9:11-Double bonds in this type of structure can be hydrogenated with

⁶ Sewell, Turnbull, and Wilson, *J.*, 1956, 4689.

⁷ Heilbronner, *Helv. Chim. Acta*, 1953, **36**, 1121.

⁸ Bladon, Henbest, and Wood, *J.* 1952, 2737.

⁹ Bentley, Henry, Irvine, Mukerji, and Spring, *J.*, 1955, 596; Henry, Irvine, and Spring, *J.*, 1955, 1607.

¹⁰ (a) Barton, *J.*, 1951, 1444; (b) Bentley, Henry, Irvine, and Spring, *J.*, 1953, 3673.

¹¹ (a) Cavalla, McGhie, and Pradhan, *J.*, 1951, 3142; (b) Dawson, Halsall, and Swayne, *J.*, 1953, 590.

¹² Holker, Powell, Robertson, Simes, and Wright, *J.*, 1953, 2414.

¹³ Barton, Fawcett, and Thomas, *J.*, 1951, 3147.

some difficulty,^{14, 15} and are stable to acids, although they appear to be easily epoxidised.^{10b} Oxidation of nyctanthic acid with chromic acid on a small scale afforded fractions having strong ultraviolet absorption bands [λ_{max} , 250 (ϵ 6000) and 259 m μ (ϵ 3500)]; oxidation of lanost-7-ene derivatives has yielded¹¹ 7-oxo- $\Delta^8(9)$ -compounds having absorption bands in the same region [λ_{max} , 255 m μ (ϵ 12,000)]. Triterpenoid acids are generally sterically hindered, their esters being hydrolysed only slowly by alkali [*e.g.*, pinicolic acid A (II)¹⁶]. However, the esters of polyporenic acid A (III)¹⁷ and dihydropolyporenic acid,⁵ like methyl nyctanthate, are hydrolysed fairly readily. The carboxyl group of nyctanthic acid therefore probably occupies a terminal position in the side-chain. As the side-chain also contains a vinylidene group, and the acid is neither $\alpha\beta$ -unsaturated (absence of ultraviolet absorption bands) nor $\beta\gamma$ -unsaturated [is not decarboxylated at the m. p.; cf. polyporenic acid A (III)^{5, 18}], only a limited number of side-chain structures are possible. The presence of a $>\text{CH}\cdot\text{CO}_2\text{H}$ or $\text{CH}_2\cdot\text{CO}_2\text{H}$ group was confirmed by converting methyl nyctanthate into the corresponding diphenylcarbinol; this was dehydrated to a diphenylethylene, which had the expected ultraviolet light absorption. Nyctanthic acid formed a cyclohexylammonium salt, which readily lost the amine, for example on gentle heating; this behaviour recalls that of polyporenic acid A.¹⁷ An attempted Oppenauer oxidation of nyctanthinol yielded no aldehyde. The properties of nyctanthic acid are consistent with those of a tetracyclic triterpenoid having the partial structure (IV): the absence of a hydroxyl group (or oxo group) is remarkable. Experiments are in progress on its conversion into the parent hydrocarbon.

EXPERIMENTAL

$[\alpha]_D$ refer to CHCl_3 solutions at $20^\circ \pm 2^\circ$. Analytical samples were dried at $100^\circ/0.01$ for 3 hr. Solutions in ethanol ("P.I. rectified spirit") were used for the ultraviolet light-absorption measurements with a Unicam SP. 500 spectrophotometer. Infrared spectra were measured by the potassium bromide disc technique. Alumina for chromatography was activated by acid treatment,¹⁹ and had an activity of II (Brockmann and Schodder²⁰).

Isolation of Nyctanthic Acid.—Seed kernels of *Nyctanthes arbor-tristis* from the Benares district were dried, crushed, and extracted with light petroleum (b. p. 40 — 60°) in a continuous extractor for 24 hr. Removal of the solvent gave a pale yellow-brown oil in 12—16% yield. After several weeks at 0° , this deposited crystals; one recrystallisation from ethanol gave fairly pure nyctanthic acid, as plates, m. p. 222 — 223° ; the further purified product had m. p. 222.5 — 223.5° , $[\alpha]_D +86^\circ$ [Found: C, 81.8; H, 10.7%; equiv. (by alkali titration in aqueous ethanol), 428; *M* (Rast), 400; *M* (Zerewitinoff), 450. $\text{C}_{30}\text{H}_{48}\text{O}_2$ requires C, 81.75; H, 11.0%; *M*, 441. $\text{C}_{28}\text{H}_{46}\text{O}_2$ requires C, 81.65; H, 10.85%; *M*, 427]. The yield of acid was 0.07—0.9% of the crude oil, and seemed to vary widely with crops harvested in different years.

β -Sitosterol.—The oil (200 g.) from which nyctanthic acid had been removed was boiled for 2 hr. with 10% methanolic potassium hydroxide (500 c.c.). Much of the methanol was distilled off, water added, and the mixture extracted with ether, to yield a syrup (7.5 g.). This was chromatographed on alumina (10×2 cm.); benzene-ether (8:1) eluted a solid (0.75 g.), m. p. 132 — 136° . Two crystallisations from ethanol afforded β -sitosterol, m. p. 139 — 140° , $[\alpha]_D -33^\circ$; the acetate had m. p. 128 — 130° , $[\alpha]_D -42^\circ$ (Found: C, 81.3; H, 11.5. Calc. for $\text{C}_{31}\text{H}_{52}\text{O}_2$: C, 81.5; H, 11.45%), and the benzoate, m. p. 147° , $[\alpha]_D -8^\circ$.

Properties of Nyctanthic Acid.—The acid did not react with acetic anhydride-pyridine, with digitonin solution, or with 2:4-dinitrophenylhydrazine solution. It was strongly absorbed on alumina and could not be eluted with ordinary solvents. Warm aqueous sodium hydroxide yielded an insoluble sodium salt, which did not melt below 300° and regenerated nyctanthic acid with mineral acids. Nyctanthic acid (105 mg.) in chloroform (100 c.c.) was boiled for 2 hr. in a current of dry hydrogen chloride. Removal of the solvent left a glass; this was boiled

¹⁴ McGhie, Pradhan, and Cavalla, *J.*, 1952, 3176.

¹⁵ Voser, Montavon, Gunthard, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 1893.

¹⁶ Guider, Halsall, and Jones, *J.*, 1954, 4471.

¹⁷ Curtis, Heilbron, Jones, and Woods, *J.*, 1953, 457.

¹⁸ Halsall, Hodges, and Jones, *J.*, 1953, 3019.

¹⁹ Lederer and Lederer, "Chromatography," Elsevier, Amsterdam, 1953, p. 21.

²⁰ Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

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with 5% methanolic potassium hydroxide; acidification yielded nyctanthic acid (97 mg.), m. p. and mixed m. p. 215—219°. The substance (50 mg.) with sulphuric acid (0.1 c.c.) in glacial acetic acid (12 c.c.) at 40° for 3 hr. was unchanged (cf. Barton and Overton²¹). Crystalline salts were obtained from nyctanthic acid and *cyclohexylamine* or 2-aminoethanol in ether; they were unstable and reverted to the free acid when heated below 200°. The *cyclohexylamine* salt lost amine in a vacuum-desiccator. The acid (45 mg.) was boiled for 1 hr. with thionyl chloride (0.2 g.) in benzene (5 c.c.). The solvent was distilled, the residue treated with ammonia in ether, and the product recrystallised from ethanol, to yield *nyctanthamide*, m. p. 174° with change of crystalline form at 153—158°, $[\alpha]_D + 90^\circ$ (Found: N, 3.55. $C_{30}H_{49}ON$ requires N, 3.2%).

The acid (1.23 g.) was treated with excess of diazomethane in ether. The crude product was chromatographed on alumina; elution with light petroleum (b. p. 60—80°) afforded a clear glass, which crystallised after considerable manipulation. Recrystallisation from methanol-chloroform gave *methyl nyctanthate* as blades, m. p. 125—126°, $[\alpha]_D + 86^\circ$ (Found: C, 81.6; H, 10.9. $C_{31}H_{50}O_2$ requires C, 81.85; H, 11.1%). The ester was hydrolysed quantitatively in 10% methanolic potassium hydroxide at 20° during 19 hr.

Nyctanthinol.—Methyl nyctanthate (386 mg.) in ether (10 c.c.) was added to lithium aluminium hydride (1 g.) in ether (100 c.c.). The mixture was refluxed for 5 hr., then decomposed cautiously with water and washed with dilute sulphuric acid. Evaporation of the ether and crystallisation from aqueous methanol yielded *nyctanthinol* as needles, m. p. 128—129°, $[\alpha]_D + 105^\circ$ (Found: C, 84.45; H, 11.8. $C_{30}H_{50}O$ requires C, 84.4; H, 11.8%). Acetic anhydride-pyridine gave the *acetate*, needles (from methanol), m. p. 102.5—104°, $[\alpha]_D + 88^\circ$ (Found: C, 81.45; H, 11.2. $C_{32}H_{52}O_2$ requires C, 82.0; H, 11.2%).

Treatment of nyctanthinol or dihydronyctanthinol with *p*-benzoquinone and aluminium *tert*-butoxide in boiling anhydrous benzene for 12 hr. did not appear to yield aldehydes, as the products gave no precipitate with 2:4-dinitrophenylhydrazine solution.

Dihydronyctanthic Acid.—Hydrogenation of nyctanthic acid (8 mg.) over Adams catalyst in acetic acid occurred rapidly. The completed uptake (15 min.) corresponded to the presence of one double bond in a molecular weight of 445, 470 (duplicate runs). The reduced product was crystallised from methanol, and had m. p. 193—194.5°. In larger scale hydrogenations, the product was recrystallised from ethanol, to yield *dihydronyctanthic acid* as needles, m. p. 194—196.5°, $[\alpha]_D + 77^\circ$ (Found: C, 81.1; H, 11.45. $C_{30}H_{50}O_2$ requires C, 81.4; H, 11.4%). The methyl ester was obtained, by using diazomethane, only as a colourless glass which could not be purified (Found: C, 80.75; H, 10.3. Calc. for $C_{31}H_{52}O_2$: C, 81.5; H, 11.45%).

Dihydronyctanthinol.—Quantitative microhydrogenation of nyctanthinol over Adams catalyst in acetic acid established the presence of one reactive double bond in a molecular weight of 420, 440, 440, 460 (replicate determinations). Reduction on a larger scale gave *dihydronyctanthinol* as needles (from methanol), m. p. 152.5—153°, $[\alpha]_D + 90^\circ$ (Found: C, 83.7; H, 12.1. $C_{30}H_{52}O$ requires C, 84.05; H, 12.25%). The acetate had m. p. 117.5—119°, $[\alpha]_D + 71^\circ$, but was apparently impure (Found: C, 80.65; H, 11.9. Calc. for $C_{32}H_{54}O_2$: C, 81.6; H, 11.55%).

Ultraviolet End-absorption Measurements.—Cholesterol, β -sitosterol, and 3 β -acetoxyergosta-7:22-diene gave figures almost identical with those reported by Bladon, Henbest, and Wood.⁸ Nyctanthinol had ϵ 350 at 220 μ , 1700 at 215 μ , and 4300 at 210 μ ; dihydronyctanthinol had ϵ 300 at 220 μ , 1250 at 215 μ , and 2800 at 210 μ .

Reaction of Methyl Nyctanthate with Phenylmagnesium Bromide.—The ester (210 mg.) in ether (10 c.c.) was refluxed for 3 hr. with an excess of ethereal phenylmagnesium bromide. The mixture was decomposed with aqueous ammonium chloride, then washed with water, and the solvent removed. The product was chromatographed on alumina; the *diphenylcarbinol* was a glass (190 mg.), $[\alpha]_D + 51^\circ$, λ_{max} . 252 (ϵ 1150) and 258 μ (ϵ 1130) (Found: C, 86.9; H, 10.1. $C_{42}H_{58}O$ requires C, 87.1; H, 10.1%). The alcohol (225 mg.) was boiled for 1 hr. with pyridine (15 c.c.) and phosphorus oxychloride. Chromatography on alumina and elution with light petroleum gave a colourless glass (136 mg.) which had the light-absorption properties of a *diphenylethylene* [λ_{max} . 252 μ ($\log \epsilon$ 4.15)] (Found: C, 89.75; H, 10.15. $C_{42}H_{58}$ requires C, 89.9; H, 10.1%). Shoppee and Stephenson²² give λ_{max} . 253 μ , $\log \epsilon$ 4.2, for a steroid diphenylethylene.

²¹ Barton and Overton, *J.*, 1955, 2639.

²² Shoppee and Stephenson, *J.*, 1954, 2230.

[1957]

A Total Synthesis of (\pm)-Ferruginol.

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The authors are indebted to Professor M. Stacey, F.R.S., for advice and encouragement, to Dr. D. H. Whiffen for infrared absorption measurements, and to the British Council for a grant (to S. K. V.).

CHEMISTRY DEPARTMENT, UNIVERSITY OF BIRMINGHAM,
EDGBASTON, BIRMINGHAM, 15.

[Present address (S. K. V.): COLLEGE OF SCIENCE,
HINDU UNIVERSITY, BANARAS, 5, INDIA.]

[Received, September 13th, 1956.]
