CONSTITUTION OF THE COLOURING MATTER OF *NYCTANTHES ARBORTRISTIS*.

IDENTITY OF NYCTANTHIN WITH α-CROCETIN.

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(*Communicated by Dr. S. Dutt.*)

(*Received January 21, 1936.*)

Nyctanthes arbortristis Linn. known as '*Harsinghar*' in Hindustani and as '*Shieuli*' in Bengali is a plant of the natural Order of Oleaceæ. It is a large shrub with rough leaves and sweet-scented flowers occurring in the sub-Himalayan and Terai tracts as well as in Central India, Burma, and Ceylon. The flowers open towards the evening and fall to the ground on the following morning. The corolla tubes are orange coloured and are well-known to give a beautiful but fleeting dye which still finds limited application for dyeing silk in Northern India.

Hill and Sirkar (*J.C.S.*, vol. 91, p. 1501; 1907) examined these flowers and reported the isolation of a red crystalline colouring matter termed by them *nyctanthin*, which they isolated by repeatedly extracting with boiling alcohol the precipitate obtained by gently warming for several hours an aqueous decoction of the flowers with 1 per cent hydrochloric acid. The alcoholic extract on again heating with 1 per cent hydrochloric acid deposited the dye in red flocks which were finally crystallized from pyridine or phenylhydrazine. Nyctanthin separated from pyridine in minute regular hexagons and from phenylhydrazine in rhombic crystals, which were yellow while wet and brick red when dry, and according to Hill and Sirkar melted at 234°–235°. With sulphuric acid it gave an intense blue colour, which rapidly turned yellow.

The analyses of nyctanthin by Hill and Sirkar agreed closely with $C_{20}H_{27}O_4$ or $C_{15}H_{20}O_3$ but of these formulæ the former was considered preferable, although it contained an odd number of hydrogen atoms. They also reported the isolation of a mono-acetyl derivative $C_{20}H_{26}O_4(COCH_3)$ but did not record its melting point.

A. G. Perkin (*J.C.S.*, vol. 101, p. 1539; 1912) isolated in 0·1% yield from the flowers of '*Cedrela toona*' or the Indian Mahogany by acid hydrolysis of an aqueous extract of the flowers and subsequent crystallization from pyridine, a red dye-stuff melting at 285°–287° (corr.) and giving with con. sulphuric acid a deep indigo-blue colour, which however rapidly turned yellow. This compound was found to be identical with nyctanthin, the colouring matter of *Nyctanthes arbortristis* to which, as Perkin observed, the previous workers

had erroneously assigned the melting point 234°-235°. He came to the conclusion that nyctanthin is closely related to bixin, the colouring matter of Annatto, *Bixa orellana*. His results indicated that the simplest formula for nyctanthin should be $C_{15}H_{18}O_3$ (Mean C=72·68%, H=7·7%).

'Among the numerous formulæ then proposed for bixin that of Van Hasselt is $C_{29}H_{34}O_5$ and should this eventually prove to be correct then nyctanthin is possibly an oxy- or hydroxy bixin $C_{29}H_{34}O_6$ (requiring C= 72·8; H=7·71%)'.[1]

'The colouring matter of the flower of Indian Mahogany, *Cedrela toona*, which was isolated by A. G. Perkin (*J.C.S.*, vol. 101, p. 1538; 1912) and identified as Nyctanthin from *Nyctanthes arbortristis* (Oleaceæ) obtained by E. G. Hill (*J.C.S.*, vol. 91, p. 1501; 1907) is *presumably* (vermuthlich) identical with α-crocetin. (M.P. 285–287. C, 72. 69; H, 7·75%; deep blue colouration in conc. sulphuric acid, which soon changes to brown; description of the pyridine salt, reduction with zinc dust in alkaline solution).'[2]

With a view to isolate the glucoside of nyctanthin, the possible existence of which is evident from the method of isolation followed by Hill and Sirkar and by A. G. Perkin and also to elucidate the structure of nyctanthin, the present investigation was undertaken. From the benzene extract of the dried flower stems, 6·1% of a non-drying oil having 20°=0·9178, sap. value 178·5, acid value 3·14, and iodine value 96·32 and from the alcoholic extract 6·2–6·4% of *d*-mannitol (Hill and Sirkar, *l.c.*) melting at 166° have been isolated. The pure colouring matter was isolated by an elaborate method of purification described in the experimental part of the paper and melted at 272°-273° (uncorr.).

On analysis it gave results agreeing with the formula $C_{20}H_{24}O_4$ which is the same as that of α-crocetin (R. Kuhn and F. L'Orsa, *Ber.*, vol. 64, (B), p. 1732 (1931); P. Karrer et al., *Helv. Chim. Acta*, vol. 15, p. 1399 (1932). The melting point of α-crocetin according to Karrer is 272°-273° (uncorr.) and 283°–285° (corr.), and it is interesting that the melting point of nyctanthin recorded by A. G. Perkin is 285°–287° (corr.). The melting point of nyctanthin was found to remain undepressed by admixture with α-crocetin obtained through the kindness of Prof. P. Karrer. Thus the conclusion seems to be irresistible that nyctanthin is identical with α-crocetin and exists free to the extent of about 0·1% in the flower stems, although it is possible that it may be present as a glucoside, which was hydrolyzed by an enzyme during the course of collection of the flower stems. Nyctanthin obtained either by the method described in the experimental portion or by the method of Hill and Sirkar (*loc. cit.*) is consequently $\alpha\epsilon\chi\xi$ tetra-methyl tetradecaheptaene $\alpha\xi$ dicarboxylic acid as this is the constitution assigned to α-crocetin by Karrer

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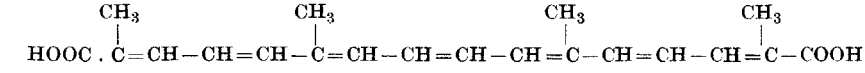
[1] Perkin and Everest, The Natural Organic Colouring Matters, 1918, p. 615.

[2] Kuhn, Winterstein and Wiegand, *Helv. Chim. Acta*; vol. XI, p. 717 (1928).

and his co-workers (*Helv. Chim. Acta*, vol. 15, p. 1399 (1932) ; vol. 13, p. 707 (1930) ; vol. 16, p. 337 (1933) ; as the result of exhaustive degradation study of α-crocetin and its dimethyl ester as well as due to the remarkable synthesis of perhydro α-crocetin (*Helv. Chim. Acta*, vol. 16, p. 297 (1933).



α Crocetin

Isolation of an amorphous colouring matter is also recorded.

Experimental.

Dried flower stems free from white petals were obtained from Delhi and were coarsely powdered and extracted in 40 g. lots in a Soxhlet's apparatus with various solvents. The solvent was subsequently evaporated and the extract thus obtained dried to a constant weight at 100°. The results are given below :—

Benzene Extract (6·12%).—Light orange yellow oil.

Chloroform Extract (8·0%).—Orange yellow fatty matter, sparingly soluble in boiling alcohol and greasy to touch.

Ethyl Acetate Extract (35·2%).—Orange red mass containing large amount of tiny silky white needles identified after crystallization from boiling alcohol to be *d*-mannitol. Contained reducing sugars.

Alcoholic Extract (1·2%).—Light orange in colour containing reducing sugars but little mannitol.

Isolation of Mannitol.—For complete examination 4 kgs. of the coarsely powdered stems were repeatedly extracted with benzene in lots of 1,700 g. in a 5-litre extraction flask until no more oil was extracted. The combined orange-yellowish extracts were distilled and the orange-yellowish, fragrant, tasteless non-drying oil was collected. The oil-free powder was successively extracted with rectified spirit till the extracts were coloured only pale yellow. The orange-yellow extracts were filtered hot and the combined extracts were distilled until most of the solvent had been recovered. It was then allowed to stand for a week by which time a large amount of white crystalline mass had separated. It was filtered at the pump and the residue washed with alcohol, until it had assumed a lemon-yellow colour. After two crystallizations from boiling alcohol white glistening needles melting at 165°-166° were obtained. The substance was identified as *d*-mannitol by its physical and chemical properties and also by its mixed melting point with Merck's pure mannitol.

(Found C=39·40, H=7·65 ; $C_6H_{14}O_6$ requires C, 39·55% ; H, 7·69%.) The hexa acetate prepared by the action of acetic anhydride and sodium acetate on the substance melted at 119° which is also the melting point of *d*-mannitol hexa acetate.

Isolation of Nyctanthin.—The alcoholic filtrate was concentrated first at ordinary pressure and finally under reduced pressure and was then extracted with benzene to remove oily and waxy matter. It was then extracted with ether, chloroform, ethyl-acetate and acetone in succession but from none of them could any crystalline substance be isolated. Therefore the whole of the coloured extract was dissolved in alcohol and treated with a slight excess of hot alcoholic lead acetate, when first a pale yellow and finally an orange precipitate separated. The lead lake was filtered and well washed with hot alcohol and water. This on decomposition with pure sulphuretted hydrogen in alcoholic suspension and subsequent filtration of the lead sulphide gave a deep brownish yellow solution, which on concentration deposited no crystalline stuff and gave with con. sulphuric acid a light indigo blue colour, which however rapidly turned brown and then yellow. It was insoluble in cold or hot benzene, chloroform, ethyl acetate, carbon tetra-chloride, ether and petroleum ether and was soluble in ethyl and methyl alcohol. On repeated extraction with benzene, chloroform and ethyl acetate no crystallizable substance could be isolated. The residue left after exhaustive extraction was a pale yellow amorphous mass giving a green colour with alcoholic ferric chloride and reduced Fehling's solution on heating and was evidently a tannin.

The alcoholic filtrate and washings from the precipitate with lead acetate was dissolved in distilled water and gave a bright yellow precipitate with excess of basic lead acetate. The precipitate was well washed with hot water and, on decomposition with pure sulphuretted hydrogen in alcoholic suspension and after filtering off the lead sulphide, gave an orange red solution, which was kept over-night with dry lead carbonate in order to remove the excess of hydrogen sulphide. The filtered lead sulphide still retained a considerable quantity of the dye-stuff, and after it had been dried in air at ordinary temperature, was extracted several times with boiling alcohol and the filtrate concentrated and mixed with the main solution. The orange-red solution on concentration and cooling slowly deposited a bright yellow crystalline mass, which was filtered and washed at the pump and the filtrate on further concentration gave a little more of the dye-stuff. This dye-stuff, after several crystallizations from boiling alcohol, was obtained pure and after drying at 120° in air-oven melted at 272°-273° (uncorr.); mixed with pure α-crocetin it melted at the same temperature. It is insoluble in water, benzene, carbon-di-sulphide, ether, chloroform, carbon-tetrachloride and acetone and very soluble in pyridine. From the latter solvent it crystallized in glistening crystals, which after washing with benzene in order to remove pyridine were dried in air-oven at 120° and melted sharp at 272°-273° (uncorr.).

(Found C, 73·01, 72·98; H, 7·47, 7·39; $C_{20}H_{24}O_4$ requires C, 73·14%; H, 7·34%.)

Potassium Salt of Nyctanthin.—Nyctanthin (·5 g.) was dissolved in a minimum amount of 1 per cent aqueous caustic potash and to the resulting solution 20 per cent caustic potash was gradually added till no further pre-

cipitation took place. It was filtered at the pump and well washed with cold alcohol and purified by crystallization from hot ethyl alcohol, when it separated as yellowish red nodular aggregates, readily soluble in water to deep yellow solution and sparingly soluble in cold alcohol. It was dried in the steam oven and analyzed.

(Found C=59·24, H=5·56, K=19·91. $C_{20}H_{22}O_4K_2$ requires C, 59·37%; H, 5·44; K. 19·34.)

Dimethyl ester of Nyctanthin.—To the ice cooled solution of nyctanthin (·3 g.) dissolved in absolute alcohol (200 c.c.), a solution of diazomethane in absolute ether obtained from 3 c.c. of nitroso methyl-urethane was added in the course of half an hour. The mixture was then allowed to remain at room temperature for a few hours to allow the liberation of nitrogen to become complete. On concentrating the solution to a small volume, dimethyl ester separated as a red crystalline powder, which after recrystallization melted at 212°-213°C.

(Found C, 73·82; H, 804. $C_{22}H_{28}O_4$ requires C, 74·12%; H, 7·92%.)

The alcoholic mother liquor gave on complete evaporation of the solvent a brownish-yellow amorphous stuff, which was successively extracted with carbon-disulphide, chloroform and finally with ethyl acetate, which removed any remaining trace of α-crocetin that was still present. The residual stuff after drying in vacuum melted at 103-104°C. and dissolved in ammonium and alkali hydroxides and carbonates to deep yellow solutions; it gave with con. sulphuric acid a deep blue colour which gradually turned violet, red and then brown; it gave with alcoholic ferric chloride a deep brownish red colour and then a precipitate, with alcoholic lead acetate a brownish red precipitate, and reduced Fehling's solution only after hydrolysis.

All attempts to crystallize it were unsuccessful.

(Found C=52·18, 52·29; H=6·34, 6·39. $C_{17}H_{24}O_{10}$ requires C, 52·6%; H, 6·2%; M=388. $C_{20}H_{28}O_{21}$ requires C, 52·2%; H=6·1%; M=460.)

The aqueous filtrate and washings from the basic lead lake were freed from lead in the usual way and the resulting solution contained glucose in large amount besides mannitol, since it formed an osazone melting at 202°, which was identified as phenyl glucosazone.

The author wishes to express his indebtedness to the Kanta Prasad Research Trust of the Allahabad University for a scholarship which enabled him to undertake this investigation and to Dr. S. Dutt, D.Sc., for his keen interest in the work and for correcting the manuscript. The author wishes to convey his heartiest thanks and indebtedness to Prof. Dr. P. Karrer of Zürich University for a sample of α-crocetin and for valuable information.