

CHEMICAL INVESTIGATION OF NEEM-SEEDS

A.M. AHSAN AND GEORGE HAHN

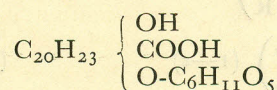
Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

In view of the enormous importance attached to the therapeutic properties of the various parts of the Neem-tree (*Azadirachta Indica*, A. Juss.) in the indigenous systems of medicine, and also of the fact that fairly extensive studies in their chemical constituents carried out by earlier authors¹⁻⁶ failed to yield any well-defined products to which these properties could be attributed, it was considered of interest to re-investigate the seeds and the oil for their bitter and sulphur-containing constituents.

The method of isolation evolved after preliminary experiments consisted in the fractional precipitation of the oil with increasing quantities of petroleum ether. Further, the alcoholic extract of the seed-cake was worked up generally following the mild procedure employed by Siddiqui^{7,8} in the isolation of Nimbin and other bitter constituents. Thus on removal of the residual oil from the semi-solid alcoholic extractive with petroleum ether, and repeatedly digesting the residue with water, a water-insoluble bitter principle was obtained in the form of a light-brown amorphous powder, which, alongwith the precipitates referred to above, served as starting materials for the present study. Details of the process followed in the isolation of the various constituents are given in the experimental. As a result of this work a series of products, constituents A, B₁, B₂, C and D, have been isolated, which are noted in Table I alongwith data relating to the crystalline products reported by earlier authors which were not, however, obtained in the present procedure of isolation. The table also includes the data for a sulphur containing oily constituent.

Of the two constituents B₁ and B₂, it will be of interest to note that while they have the same melting points and analytical data, one of them is bitter and the other non-bitter. Their optical rotations also vary. In respect of B₁ it has been further noted that it reduces Fehling's solution only after boiling with a few drops of hydrochloric acid, which might indicate a glycosidal structure for it, with C₂₁H₂₆O₄ as the aglycone residue after accounting for the sugar moiety. Moreover the presence of a carboxyl group was indicated by the hydroxamic acid test, and the phenol grouping by Millon's reagent and paranitroaniline. The deep red colour of the solution in 2N sodium hydroxide together with the decolorisation of permanganate solution in the cold

indicates a dihydroxy aromatic system. Considering the fact that the glycoside goes into solution in dilute ammonia and sodium carbonate solution indicating the presence of a carboxyl group, it is tentatively, suggested to represent it as follows :—



Further the two crystalline constituents B₁ and B₂ react with 2N sodium hydroxide to give a deep red solution. As all the petroleum ether precipitates and the alcoholic extractive of the cake give the same coloration with 2N sodium hydroxide, it would appear that much larger quantities of the above crystalline constituents occur in the seeds than it has been possible to isolate.

In the course of this work, it was observed that the oil from the seed crop of 1956 yielded a negligible amount of precipitate on treatment with petroleum ether. This recalls the observation of Siddiqui et al. that they were not able to isolate the two crystalline bitters, Nimbin and Nimbinin, from certain fresh samples of solvent-extracted Neem oil and that their yields from various commercial samples varied appreciably.⁹ It would thus appear that the nature and yields of the constituents of the Neem seed and oil are liable to variations due to soil and climatic conditions, and other factors connected with the harvesting and storage of the seeds.

Experimental

5.6 kg. of Neem-kernels, pressed out in the oil mill, yielded 1500 cc. of oil and 3.2 kg. of cake.

Treatment of the Oil

The oil (1500 cc.) was successively treated with 375 cc. portions of petroleum ether (b.p. 60-80°C.). Each time after adding the solvent the oil was warmed to 50°C. for 15 minutes to flocculate the precipitate formed, and then allowed to stand for 24 hours. The precipitate was then filtered and washed with petroleum ether till free from oil. Any loss of solvent due to evaporation was accounted for in the addition of the next 375 cc. lot of petroleum ether, and the whole process repeated. After 5 such operations no more pre-

cipitate was obtained on further addition of petroleum ether.

However, on standing for a month a substantial quantity of a deposit was obtained. On subsequent addition of petroleum ether and standing no further precipitation was observed. Seven separate fractions were thus obtained, the characterisation of which is given in Table II.

Isolation of constituent A.—Precipitate 2 (1.8 g.) was dissolved in benzene (10 cc.) filtered from a negligible quantity of insoluble material, and the solution concentrated in vacuo to a fluid of thin syrupy consistency. On standing in the cold a crystalline substance was obtained which was sucked off and washed with a few drops of cooled benzene (20 mg.). On recrystallisation from chloroform and alcohol constituent A (8 mg.) was obtained in the form of colourless clusters of tapering rods (Plate I) which melted

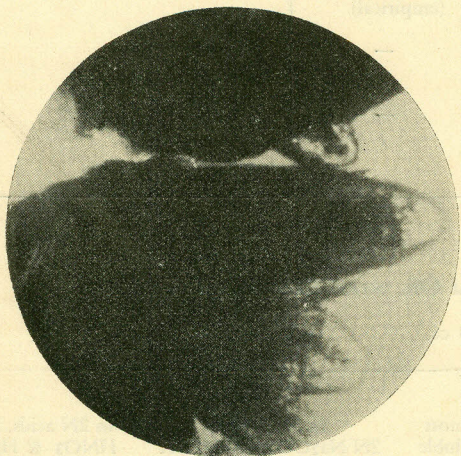


Plate I.—Constituent A.

sharply at 95°C. The substance was readily soluble in chloroform, ethyl acetate and benzene, fairly so in alcohol and acetone, almost insoluble in ether and petroleum ether. It was found to contain no sulphur.

Isolation of constituents B₁ and B₂.—Precipitate 6 (23 g.) when treated with alcohol (100 cc.) went into solution. This immediately gave a white precipitate which was filtered, washed with alcohol and dried (2.1 g.). It was dissolved in chloroform (10 cc.) and alcohol (3 cc.) was added to the solution, which was then concentrated in vacuo at 50°C. till faintly turbid. On cooling in ice a crystallisate was obtained. The filtrate and washings from it yielded on con-

centration in vacuo and cooling, a second crop of crystals. On repeated fractional crystallisation in this manner, B₁ was finally obtained from the tail fractions in the form of wedge-shaped crystals 0.75 g., (Plate II), which darkened at 200°C.



Plate II.—Constituent B₁.

and melted at 307°C. (decomp.). It was readily soluble in chloroform and glacial acetic acid, fairly so in acetone and ethyl acetate, sparingly soluble in alcohol and nearly insoluble in water, benzene, ether and petroleum ether. $[\alpha]_D^{20} = +152^\circ$ in 0.1% solution in alcohol. $[\alpha]_D^{31.5} = +156^\circ$ in 1% chloroform solution.

Calculated for C₂₇H₃₆O₉ (505): C, 64.28; H, 7.1; O, 28.57. Found, after drying in vacuo at 50°C.: C, 64.45; H, 7.01; O, 28.33.

B₁ dissolved to a yellow solution on heating in 2N sodium carbonate. In concentrated ammonia it slowly went into solution on standing. With 2N sodium hydroxide, however, it readily yielded a yellow solution, from which the starting material could be got back on immediate acidification. On longer standing the colour deepened to red and B₁ could no more be recovered from the solution. Instead a bright yellow amorphous substance soluble in alcohol was obtained which could not be crystallised. With concentrated hydrochloric acid there was no reaction in the cold. 2N sulphuric acid dissolved the substance slowly on heating, and the concentrated acid gave with it a reddish-coloured solution. Addition of water produced a white flocculent precipitate from the latter. Potassium permanganate solution was decolorised immediately. Fehling's solution was not reduced by an alcoholic solution of B₁. When, however, the latter was boiled with a little hydrochloric acid it readily reduced Fehling's

TABLE I.—CRYSTALLINE OR OTHERWISE CHARACTERISED CONSTITUENTS OF NEEM.

No.	Name	M.P. °C.	B.P. °C.	$[\alpha]_D$	Taste	Molecular formula	Isolated by	Literature
1.	Margosopicrin ..	221-222	—	+163.8°	Bitter	C ₂₄ H ₃₂ O ₈	Watson, Chatterjee and Mukherjee	5
2.	—	Does not melt below 230	—	-325°	Not bitter	C ₁₃ H ₂₄ O ₇ S ₂		
3.	Neemola (Oil) ..	—	156—158 at 118mm.	—	Not bitter	C ₁₅ H ₃₀ O ₃ S	Qudrat-i-Khuda, Ghosh and Mukherjee	6
4.	Nimbin ..	205	—	+170°	Bitter	C ₂₈ H ₄₀ O ₈	Siddiqui	7,8
5.	Nimbinin ..	192	—	—	Bitter	C ₉ H ₁₀ O ₃ (empirical)		
6.	Constituent A ..	95	—	—	Not bitter	—	Present authors	
7.	Constituent B _I ..	307 (decomp.)	—	+156°	Not bitter	C ₂₇ H ₃₆ O ₉		
8.	Constituent B ₂ ..	307 (decomp.)	—	+186.4°	Bitter	C ₉ H ₁₂ O ₃ (empirical)		
9.	Constituent C ..	280 (decomp.)	—	—	Not bitter	—		
10.	Constituent D ..	260 (decomp.)	—	—	Intensely bitter	—		

TABLE II.—FRACTIONAL PRECIPITATION OF NEEM OIL WITH PETROLEUM ETHER.

Amount of Oil=1500 cc.

No. of fractions	Volume of petroleum ether added	Amount of precipitate formed in 24 hrs.	Soluble	Sparingly soluble	Almost insoluble	In dil. alkalis 2N Na ₂ CO ₃ , 2N NaOH	In 2N acids, HCl, HNO ₃ & H ₂ SO ₄
1	1 × 375 cc.	2.2 g.	Ethyl Acetate, Chloroform, Acetone, Alcohol	Benzene	Ether, Petroleum ether	Soluble in the cold	Insoluble in the cold
2.	2 × 375 cc.	1.8 g.	"	"	"	"	"
3.	3 × 375 cc.	2.5 g.	"	"	"	"	"
4.	4 × 375 cc.	2.9 g.	"	"	"	"	"
5.	5 × 375 cc.	9.6 g.	"	"	"	"	"
6.	6 × 375 cc.	23 g. (after standing 1 month)	"	"	"	"	"
7.	7 × 375 cc.	Nil (after standing 6 months)					

solution. Millon's¹⁰ and p-nitroaniline¹¹ tests for the phenolic group were positive. Test for acid grouping by conversion to hydroxamic acid¹² was also positive.

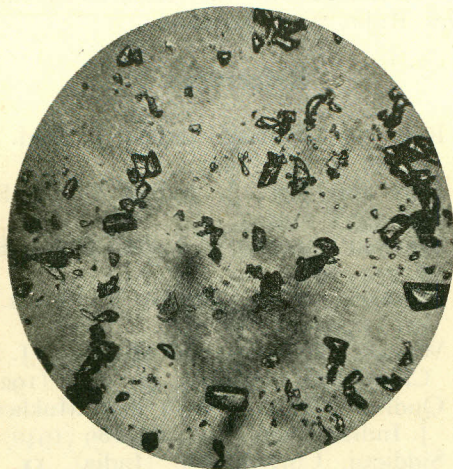


Plate III.—Constituent B₂.

Isolation of constituent B₂.—The first crop of crystals obtained from precipitate 6 was dissolved in chloroform (10 cc.), and alcohol (2 cc.) was added to the solution, which was concentrated in vacuo till a faint turbidity appeared. Repeated recrystallisation of the crystallisate obtained on cooling the concentrate finally yielded B₂ (Plate III) which was bitter to taste, had about the same solubilities as B₁ darkening from 220°C. onwards. It showed $[\alpha]_D^{28} = +186.4^\circ$ in 1 to 2% chloroform solution, as against $+156^\circ$ for B₁, the optical rotation remaining unaffected on further crystallisation.

Calculated for C₂₇H₃₆O₉: C, 64.28; H, 7.1; O, 28.57. Found, after drying in vacuo at 50°C.: C, 64.28; H, 7.40; O, 27.81.

With 2N sodium hydroxide B₂ gave a deep yellow solution from which the original substance could be recovered almost quantitatively on immediate acidification as in the case of B₁.

Precipitate 3 (2.5 g.) yielded through ethyl acetate and ether a crystallisate which appeared under the microscope to have three different types of crystals, one of them corresponding to B₁, and which indefinitely melted from 200 to 300°C. (decomp.).

Precipitate 4 (2.9 g.) and 5 (9.6 g.) when worked up as precipitate 6 yielded 0.05 g. and 0.2 g. respectively of B₁. The mother liquors containing the bulk of the material failed to give any uniform crystalline product.

Treatment of the Cake

Isolation of constituent C.—The cake (3.2 kg.) was percolated 5 times at room temperature with about 5 l. of alcohol for each treatment and the combined percolates were freed of the solvent in vacuo at 50°C. The brown treacly residue was exhaustively extracted with petroleum ether (b.p. 60-80°C.) and then with water. The insoluble product, which formed a yellowish-brown powder failed to yield a crystallisate. The petroleum ether extract gave on standing for about a month at room temperature a cream-coloured precipitate from which a small quantity of B₁ could be crystallised out through alcohol. The ethereal filtrate was freed of the solvent in vacuo at 50°C. and the oily residue allowed to stand for a month, when a precipitate developed, which on crystallisation through alcohol gave white, tasteless, cauliflower-like aggregates of crystals (30 mg.) melting at 280°C. (decomp.) (Plate IV). This substance was sparingly soluble in alcohol and ethyl acetate in the hot, nearly insoluble in other common organic solvents and in water.

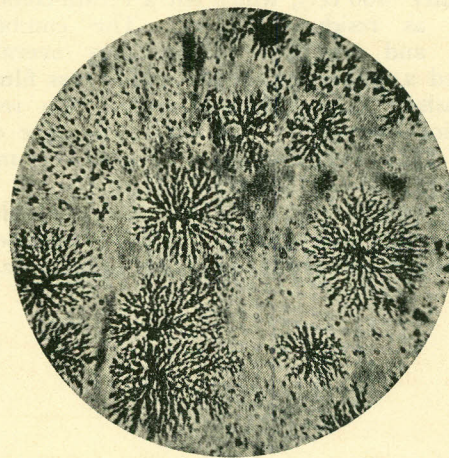


Plate IV.—Constituent C.

The substance did not react with 2N sodium carbonate and 2N sodium hydroxide in the cold. With 2N sulphuric, nitric and acetic acids there was no reaction either in the cold or on heating. With concentrated nitric acid it dissolved to a yellowish solution on heating.

Isolation of constituent D.—A sample of freshly expressed oil from the crop of seeds of 1954 (430 cc.) was filtered clear of suspensions and allowed to stand at room temperature (average 20°C.) for a fortnight. The precipitate that developed was filtered off, and the clear oil treated with an equal volume of petroleum ether (b.p.

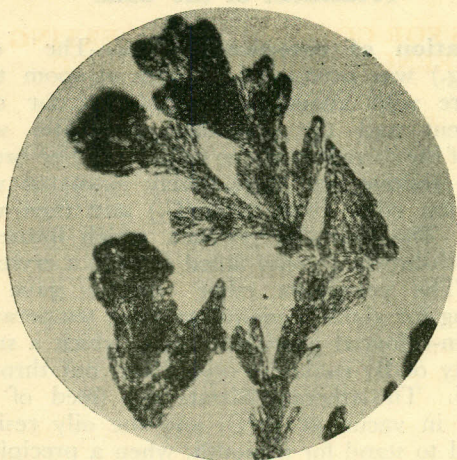


Plate V.—Constituent D.

60-80°C.) and allowed to stand overnight. The precipitate formed was filtered, washed with petroleum ether till free from oil (2.5 g.). It was then exhaustively extracted at room temperature with water (100 cc.), which left a cream-coloured powder as residue (1.5 g.). The combined filtrates and washings on standing overnight deposited a white crystalline which was filtered and washed once with cold water. On recrystallisation twice over from alcohol it gave constituent D as feather-shaped crystals (10 mg.), melting at 260°C. (decomp.) (Plate V). It was intensely bitter to taste and was readily soluble in acetone, ethyl and methyl alcohols, fairly so in ethyl acetate and almost insoluble in benzene.

Acknowledgement

The micro-analyses have been carried out by A. Bernhardt, Mikro-analytisches Laboratorium in Max-Planck-Institut für Kohlenforschung, Mulheim, Ruhr, West Germany.

References

1. Roy and Chatterji, *Indian J. Med. Research*, **5**, 656 (1917).
2. Dutt and Roy, *J. Indian Chem. Soc.*, **48**, 333T (1929).
3. Sen and Banerjee, *J. Indian Chem. Soc.*, **8**, 773 (1932).
4. Sheshadri et al., *Indian J. Pharm.*, **2**, 201 (1940).
5. Watson, Chatterjee and Mukerjee, *J. Soc. Chem. Ind. (London)*, **42**, 387T (1923).
6. Qudrat-i-Khuda, Ghosh and Mukherjee, *J. Indian Chem. Soc.*, **17**, 189 (1940).
7. Siddiqui, *Current Sci. (India)*, **11**, 278 (1942).
8. Siddiqui, *Indian Patent No.* 33,649 (1947).
9. Siddiqui, Rao and Mitra, *J. Sci. Ind. Research (India)*, **12B**, 152, (1953).
10. Fiegl, *Spot Tests* (Elsevier Publishing Co., London, 1954) 4th Edition, Vol. II Organic Applications, p. 134.
11. Clarke, *A Handbook of Organic Analysis* (Edwin Arnold and Co., London, 1926), 4th Edition, p. 83.
12. Fiegl, *Spot Tests* (Elsevier Publishing Co., London, 1954), 4th Edition, Vol. II, Organic Applications, p. 140.