INVESTIGATIONS ON ANDROGRAPHIS PANICULATA NEES

Part I.—Preliminary Examination of Some Constituents of the Leaves

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From the petroleum ether extract of the leaves of *Andrographis paniculata* Nees an α,β-unsaturated lactone, homoandrographolide ($C_{22}H_{32}O_3$), a sterol andrographosterol ($C_{23}H_{38}O$), a hydrocarbon, andrographane ($C_{40}H_{82}$), a ketone, andrographone ($C_{32}H_{64}O$), a wax, panicula wax, and two different esters containing hydroxyl groups have been isolated. The wax, has been hydrolysed, though with difficulty, and the resulting fatty acid, ($C_{27}H_{55}COOH$) has been separated in the pure condition by chromatography on alumina.

The plant *Andrographis paniculata* Nees, locally known as 'kalmegh' grows abundantly in East Pakistan. It is the principal ingredient of a household medicine, extensively used in this part of the country as a remedy for various ailments. [1-4]

In view of the wide medicinal applicability of the plant, a thorough investigation of the different parts of this plant, i.e., leaves, stems and roots, was felt necessary. It may be mentioned that no detailed investigation on this plant had been undertaken hitherto. The previous work[5-8] on this plant involved the isolation and study of andrographolide from the leaves.

We have systematically examined the leaves by first extracting with petroleum ether and then with rectified spirit. Petroleum ether extracted an α,β-unsaturated lactone, a sterol, a ketone, a hydrocarbon, a wax and two oils etc. and the rectified spirit extract yielded andrographolide among several other products. The present paper describes the constituents obtained from the preliminary petroleum ether extract.

The leaf extract with petroleum ether at room temperature, on subsequent concentration and cooling, gave a solid mass, from which a ketonic substance and a hydrocarbon were obtained. The ketonic substance melted at 85°C. and was found to have the molecular formula, $C_{32}H_{64}O$. We have called it 'andrographone.' Infrared spectrum of this substance (in chloroform) showed a peak at 1718 cm.$^{-1}$ indicating that the substance is a saturated open-chain ketone.

The hydrocarbon, which we have called 'andrographane,' melted at 67-68°C. and was found to have the molecular formula $C_{40}H_{82}$. Infrared spectrum of this substance (in chloroform) did not indicate the presence of any hydroxyl, carbonyl or any other functional group.

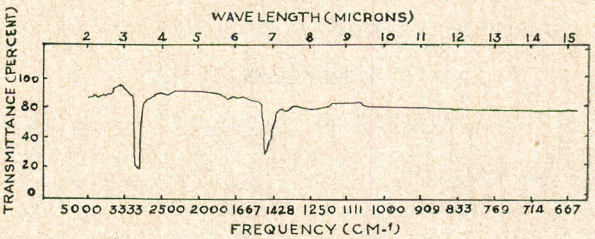

Fig. 2.—Infrared absorption spectrum of andrographane in chloroform.

The mother liquor of the solid mass, on chromatography, gave a crystalline solid melting at 115°C., a crystalline sterol, m.p. 135°C., a wax m.p. ca. 30°C. and two thick oils in addition to a further quantity of the hydrocarbon and the ketone. The solid melting at 115°C. was found to have the

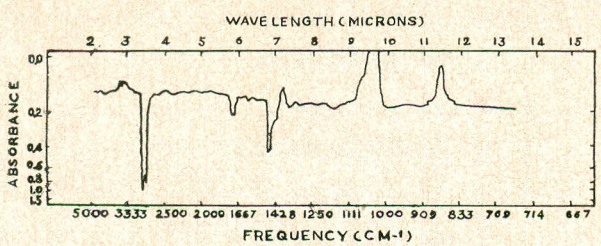

Fig. 1.—Infrared absorption spectrum of andrographone in chloroform.

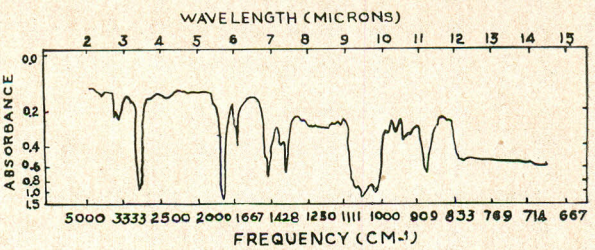

Fig. 3.—Infrared absorption spectrum of homoandrographolide in chloroform.

molecular formula, $C_{22}H_{32}O_3$. Infrared spectrum of this substance (in chloroform) showed peaks at 3496 cm.$^{-1}$ (hydroxyl), 1757 cm.$^{-1}$ (α,β-unsaturated γ-lactone), 1647 cm.$^{-1}$ ($C=CH_2$) and at 1079 cm.$^{-1}$ (higher cyclic ether). It decolourised bromine solution and responded to the Legal test for unsaturated lactones. From these findings the substance appeared to be an α,β-unsaturated γ-lactone which has been named 'homoandrographolide' by us.

The sterol responded to the Liebermann-Burchard and Salkowski tests and was found to have the molecular formula, $C_{23}H_{38}O$. Its acetyl and benzoyl derivatives had m.p. 126°C. and 143°C. respectively. It has been called 'andrographosterol' by us. It is under further examination and will form the subject matter of a separate communication. The substance melting at *ca.* 30°C. was found to be difficultly hydrolysed to an alcohol, m.p. 75°C. and an acid, $C_{27}H_{55}COOH$, m.p. 77°C. Infrared spectrum of the alcohol showed absorption peak at 3472 cm.$^{-1}$ (OH) and that of the fatty acid showed absorption at 1712 cm.$^{-1}$ (fatty acid carbonyl), 1280 and 1234 cm.$^{-1}$ (long chain fatty acid), whereas the substance melting at 30°C. showed strong absorption at, 1742 cm.$^{-1}$ (normal saturated ester carbonyl) and at 1176 cm.$^{-1}$ (C-O- stretching vibration in higher fatty acid esters). Thus the substance is a wax which we have called 'Panicula wax.'

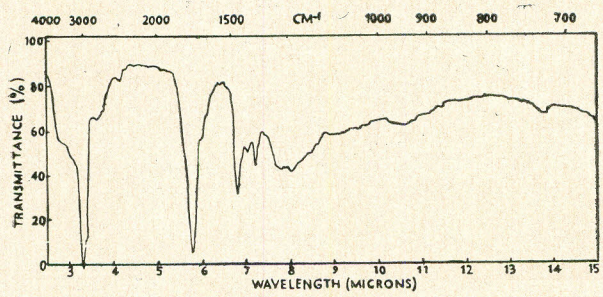

Fig. 4.—Infrared absorption spectrum of the fatty acid from 'Panicula wax' in nujol.

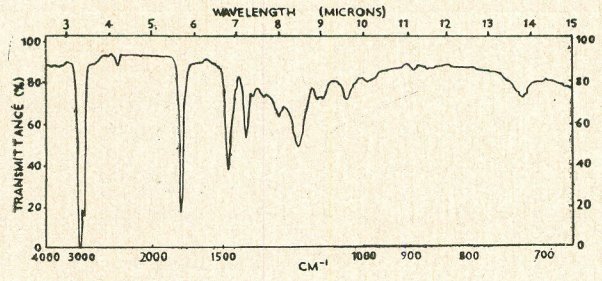

Fig. 5.—Infrared absorption spectrum of 'Panicula wax' (thin film).

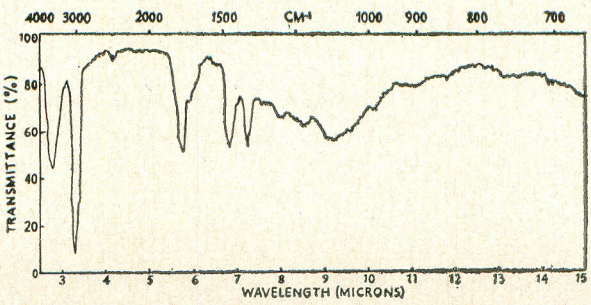

Fig. 6.—Infrared absorption spectrum of the thick oil (1) (thin film).

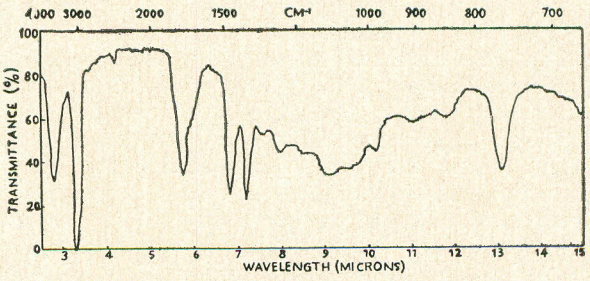

Fig. 7.—Infrared absorption spectrum of the thick oil (2) (thin film).

From the nature of their infrared spectra and absorptions at 3475, 1724 cm.$^{-1}$ and 3472, 1718 and 757 cm.$^{-1}$, the two thick oils separated by chromatography on alumina, seem to be two different esters containing free hydroxyl groups. Further work on all the above constituents separated from the petroleum ether extract is in progress.

Experimental

Brockmann neutral alumina (E. Merck) was used for absorption chromatography unless otherwise stated. Infrared spectra were determined with the help of a Perkin-Elmer infracord machine. Melting points are uncorrected. The microanalyses reported have been done by Dr. Franz Pascher, Mikroanalytisches Laboratorium, Bonn, Buschstrassc-54, West Germany and Alfred Bernhardt, Max. Plank-Institut fur Kohlenforschung, Mulheim, Ruhr, West Germany.

Isolation of a New Hydrocarbon, Andrographane.—Air-dried leaves of *Andrographis paniculata* Nees (2 kg.) were powdered and exhaustively extracted with petroleum ether (b.p. 60-80°C.) through percolation at the room temperature. The extract, on concentration and cooling, deposited a solid substance which was separated by filtration and washing with petroleum ether. The residue was redissolved in the minimum quantity of boiling petroleum ether (b.p. 60-80°C.) and the solution cooled, when a solid separated which was filtered

off. The solid residue (500 mg.) was dissolved in a hot mixture of ether and chloroform; 2 g. of alumina were added to this solution and the solvent was completely removed from it. The resulting solid mixture of the residue and alumina was placed on the top of a column of alumina. Elution of the column was first carried out with petroleum ether (b.p. 45-80°C.) and 25 fractions of 3 ml. each were collected. The first two fractions gave a negligible quantity of an ethanol-insoluble oily substance, which was rejected. The fraction (3-25) gave an almost colourless, crystalline substance (400 mg.) which on crystallisation either from ethanol or acetone did not raise the melting point. It was again chromatographed on alumina as before and then crystallised from ethanol. After three such crystallisations, the substance was finally obtained as colourless, fine, shining needles, m.p. 67-68°C. It was readily soluble in benzene and chloroform and moderately so in petroleum ether, ether, acetone, and sparingly soluble in ethanol and methanol. It did not respond to tests for unsaturation or sterols. Infrared spectrum (Fig. 2) of this substance in chloroform did not indicate the presence of any hydroxyl, carbonyl or other functional groups. This has been called 'andrographane' by us. The substance was dried by heating at 56°C. under high vacuum for 15 hours and then analysed. Found: C, 85.36; H, 14.63; mol. wt., 556; $C_{40}H_{82}$ requires: C, 85.40; H,14.59; mol. wt., 562.

Isolation of Andrographone.—After the collection of 25 fractions with petroleum ether, the alumina column was eluted with benzene and 20 fractions of 3 ml. each were collected. The fractions (1-3) gave a solid substance which melted at 70-76°C. The fractions (4-20) yielded a colourless, amorphous substance which melted at 81-83°C. It was dissolved in boiling ethanol. On cooling the solution, a flocculent precipitate appeared, which was separated by filtration. After repeating this process thrice the substance was finally obtained as a colourless, amorphous mass which melted sharply at 85°C. It was readily soluble in benzene and chloroform and moderately soluble in petroleum ether, ether, methanol, ethanol and acetone. Infrared spectrum (Fig. 1) in chloroform of this substance showed a peak at 1718 cm.$^{-1}$ which indicated that the substance is a saturated open-chain ketone.

The substance was dried under high vacuum by heating at 56°C. for 20 hours and then analysed. (Found: C, 82.63; H, 13.90; mol. wt., 428. $C_{32}H_{64}O$ requires: C, 82.83; and H, 13.90; mol. wt., 464.

The details of extraction are shown in Table 1.

TABLE 1.

No. of extraction	Solvent in litres	Time in hrs.	Andrographane in mg.	Andrographone in mg.	Mother liquor in ml.
1.	10	48	375	30	250
2.	8	48	225	20	200
3.	8	48	125	8	150
4.	8	48	75	nil	150
5.	8	48	25	nil	150
6.	8	48	nil	nil	100
	50 litres	288 hrs.	825 mg.	58 mg.	950 ml.

TABLE 2.

Sl.No. of fractions	No. of fraction	Solvent	Residue in g.	Nature of residues
(i)	1-35	Petroleum ether	5.100	Thick green mass
(ii)	36-51	-do-	1.020	Thick yellow mass
(iii)	52-61	-do-	0.075	Slightly coloured semi-solid mass
(iv)	62-70	Petroleum ether-benzene	0.750	Yellow semi-solid mass
(v)	71.85	Benzene	0.500	Light yellow crystals
(vi)	86.103	Benzene-chloroform	1.350	Light yellow thick oil
(vii)	104-122	Chloroform	1.015	Light yellow thick oil
(viii)	123-125	Methanol	0.010	Coloured impurities

The petroleum ether mother-liquor left after separation of the residue consisting of andrographane and andrographone was concentrated and chromatographed on a column of alumina. Elution was carried out successively with petroleum ether (b.p. 45-75°C.), mixtures of petroleum ether and benzene, benzene, mixture af benzene and chloroform, chloroform alone, and methanol as shown in Table 2. In all 125 fractions of 50 ml. each were collected. Depending on chemical tests, infrared spectra, melting points, and solubility etc. the residues of all the fractions were combined into eight different groups of substances os shown in Table 2 and each group was examined separately.

Preparation of Panicula Wax and Homoandrographolide.—The combined residue (5.1 g.) of fractions (1-35) was a thick, green mass which, on slow evaporation from petroleum ether solution gave fine needles together with a large quantity of a green, gummy substance. The whole mass was dissolved in petroleum ether and the solution treated with activated charcoal when a light yellow solution was obtained. The concentrated light yellow solution was chromatographed on a column of alumina. Elution was carried out with petroleum ether and mixture of petroleum ether and benzene (10:1). On removal af the solvent from the petroleum ether eluate, a thick, almost colourless oil was left as residue, which was dissolved in boiling acetone. On cooling to room temperature the solution deposited some fine needles which after separation and drying melted at 65-66°C. It was purified by repeated crystallisations first from acetone and then from ethanol. The fine white needles melted at 67-68°C. and the substance was identical with andrographane in all respects.

The acetone mother-liquor of andrographane was decolourised with activated charcoal. The colourless solution after the removal of solvent yielded a thick oil which was extracted with hot ethanol. The residual oil possessed a characteristic pleasant odour. It was insoluble in ethanol and methanol, but highly soluble in petroleum ether, ether, benzene, chloroform, acetone etc. Infrared spectrum (thin film) showed peaks at 1742 $cm.^{-1}$ (normal saturated ester carbonyl), 1176 $cm.^{-1}$ (C-O stretching vibration in higher fatty acid esters), 1032 and 720 $cm.^{-1}$ (Fig. 5). From these findings, the substance seemed to be an unusually low-melting wax which we have called 'Panicula wax.' It melted at *ca.* 30°C.

The eluate of the alumina column with petroleum ether and benzene (10:1) yielded, on removal of solvent, a light yellow residue which was dissolved in petroleum ether and filtered. The concentrated solution on standing became turbid and after some time, fine, hairy needles began to appear from it. Ultimately the mother liquor became quite clear. The crystals after separation and drying melted at 108-110°C. and did not respond to the Liebermann-Burchard test for sterols. After several crystallisations, first from methanol and then from petroleum ether, the substance was finally obtained in the form of fine, hairy, shining needles which melted at 115°C. Infrared spectrum (Fig. 3) of this substance in chloroform showed peaks at 3496 $cm.^{-1}$ (hydroxyl), 1757 $cm.^{-1}$ (α, β-unsaturated γ-lactone), 1647 $cm.^{-1}$ ($C=CH_2$) and at 1079 $cm.^{-1}$ (higher cyclic ether). The presence of the unsaturated lactone group was further confirmed by its strong response to the Legal test. The substance was highly soluble in ethanol, methanol, benzene, ether, chloroform and moderately soluble in petroleum ether. It decolourised bromine solution indicating the presence of unsaturation in the molecule.

The substance was dried by heating at 80°C. under high vacuum for 16 hours and then analysed. (Found: C, 76.68; H, 9.45; mol. wt., 305; $C_{22}H_{32}O_3$ requires: C, 76.81; H, 9.38; mol. wt., 344. We have called this lactone 'homoandrographolide.'

Examination of Fractions (36-51).—All the residues of fractions were not homogeneous. They were combined together (1:02 g.) and chromatographed on a column of alumina. Elution with petroleum ether (b.p. 45-75°C.) gave Panicula wax (0.990 g.) and that with benzene yielded the ketone, andrographone (0.025 g.).

Examination of Fractions (52-61).—The combined residues (15 g.) of fractions (52-61) was in the form of a slightly coloured semi-solid mass which was dissolved in boiling acetone. The solution on cooling deposited a colourless substance which after separation and drying melted at 83-84°C. Similar treatment with boiling ethanol raised the melting point to 85°C. The white, amorphous substance was found to be identical with the ketone, andrographone.

Examination of Fractions (62-70).—The combined residue of the fractions (62-70) were a semi-solid, yellow mass which responded to the Liebermann-Burchard[10] and Salkowski[11] tests for sterols, but could not be obtained in the crystalline condition. It was purified by repeated chromatography on alumina and subsequent repeated crystallisations from ethanol. The fine, white rectangular leaflets, thus obtained, melted at 135°C. and responded to tests for sterols. The acetate of this sterol was prepared by the action of acetic anhydride and pyridine at room temperature. It was obtained in the form of white needles that melted at 126°C.

By the action of benzoyl chloride and pyridine the benzoate of the sterol was obtained in the form of colourless needles which melted at 143°C. The sterol was dried by heating at 100°C. under high vacuum for 20 hours and then analysed. (Found: C, 83.22; H, 11.88; mol. wt., 321; $C_{23}H_{38}O$ requires: C, 83.63; H, 11.51 ml. wt., 330.

Examination of Fractions (71-85).—The combined residue (500 mg.) of fractions (71-85) spontaneously crystallised on addition of a few drops of ethanol. This after four crystallisations from ethanol gave fine, white, rectangular leaflets which melted at 135°C. These consisted of andrographosterol.

Examination of Fractions (86-103).—The combined residue (1.35 g.) of fractions (86-103) comprised a light yellow thick oil highly soluble in benzene, ether, chloroform and moderately soluble in petroleum ether but sparingly soluble in ethanol. It could not be crystallised from any solvent. Infrared spectrum (thin film) showed peaks at 3475 and 1724 $cm.^{-1}$ (Fig. 6) indicating the presence of a hydroxyl as well as an ester carbonyl group[9] in it.

Examination of Fractions (104-122).—The combined residue (1.015 g.) of fractions (104-123) was also a light yellow thick oil, very sparingly soluble in ethanol but readily soluble in benzene, ether and chloroform. Infrared spectrum (thin film) showed peaks at 3472, 1718 and 757 $cm.^{-1}$ (Fig. 7).

Fractions 123-128 were rejected.

Hydrolysis of Panicula wax.—Panicula wax (500 g.) was refluxed with 25% ethanolic KOH (7 ml.) for 48 hours and then the solvent was removed. The brown residue was treated with water and extracted thrice with chloroform. The combined chloroform extract (500 ml.), after washing with water, was dried (anhydrous $MgSO_4$). On removal of solvent a semi-solid mass was left as residue which was dissolved in petroleum ether and chromatographed on a column of alumina (15 g.). Elution was done successively with petroleum ether (b.p. 45-75°C.), benzene and chloroform and 40 fractions of 5 ml. each were collected. The fractions (1-25) was found to contain the unchanged wax (400 mg.). Fractions (26-40) gave a colourless oil (25 mg.) which solidified on the addition of methanol. It was further purified by repeating thrice the process of dissolving it in a minimum volume of benzene and precipitating with methanol when the melting point (75°C.) did not rise any more. Infrared spectrum of this substance (nujol) showed a strong peak at 3472 $cm.^{-1}$ (hydroxyl),[9] but the quantities have not yet been enough for further characterisation.

The alkaline solution was acidified with concentrated H_2SO_4 and extracted thrice with chloroform. The combined chloroform extract (175 ml.) was repeatedly washed with water until the aqueous phase was free from mineral acid and then dried ($MgSO_4$). On removal of solvent a yellow oil (40 mg.) was left as residue which was dissolved in petroleum ether and chromatographed on a column of alumina (2 g.) Elution was performed with petroleum ether and the eluate, on removal of the solvent, gave a small quantity of coloured impurities. (No effervescene was observed with sodium bicarbonate solution). The column was next eluted with benzene and the elution was continued with this solvent till the dissolution ceased. The eluate, on removal of solvent, yielded a colourless oil which solidified on addition of methanol. This was dissolved in hot methanol and the solution on cooling to room temperature deposited a white solid which after separation and drying melted at 74-75°C. By repeating this process several times the melting point was raised to 77°C. The bright, white, amorphous powder liberated CO_2 from bicarbonate solution. Infrared spectrum (Fig. 4) of this substance showed peaks at 1712 $cm.^{-1}$ (fatty acid carbonyl), 1280 and 1234 $cm.^{-1}$ (long chain fatty acid).

The substance was dried under high vacuum by heating at 56°C. for 20 hours and then analysed. (Found: C, 79.37; H, 13.081; mol. wt., 478; $C_{28}H_{56}O_2$ requires: C, 79.31; H, 13.31; mol. wt., 428.

In another experiment the hydrolysis of the wax was carried out by refluxing a mixture of the wax (2.33 g.), benzene (12 ml.)and 25% ethanolic KOH (7 ml.) for 48 hours. Only about 20% of the wax was found to be hydrolysed (cf. the process of Chibnall et. al.[12]).

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