INVESTIGATIONS ON ANDROGRAPHIS PANICULATA NEES

Part VI. The Root Flavones and Their Structures

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Abstract. Extraction of the roots of *Andrographis paniculata* with alcohol has given four flavones. Two of them, andrographin and panicolin, are shown to be 5-hydroxy-2',7,8-trimethoxyflavone and 2',5-dihydroxy-7,8-dimethoxyflavone respectively. The other two flavones are apigenin 4',7-dimethyl ether and mono-O-methylwightin. α_{I-} Sitosterol has also been isolated from the alcoholic extract of the roots.

In an earlier publication,¹ we reported two new flavones, $C_{18}H_{16}O_6$, m.p. 190–191°C and $C_{17}H_{14}O_6$, m.p. 263–264° isolated from the roots of *Andrographis paniculata*. These flavones were named andrographin and panicolin respectively. Subsequently, Govindachari *et al.*² in their investigation on the roots of the same plant isolated apigenin 4′,7-dimethyl ether(I) and mono-*O*-methylwightin(II), but they seem to have missed andrographin and panicolin. It was, therefore, considered necessary to undertake a reinvestigation of the roots. Furthermore, it was necessary to determine the structures of andrographin and panicolin. This paper deals with the results of these investigations.



The alcoholic extract of the dried roots, on successive concentration and cooling, afforded four crops of crystals. The first three crops were a mixture of several components, while the fourth crop consisted mainly of panicolin.¹ The first three crops appeared very similar and, therefore, they were combined redundent and taken up in chloroform. The residue, redundent after removal of solvent from the chloroform solution, was extracted with petroleum ether to give (a) petroleum ether soluble and (b) petroleum ether insoluble fractions. The petroleum ether soluble fraction furnished a colourless crystalline substance, m.p. 164° which responded to Lieberman-Burchard test.³ It gave a crystalline acetate, m.p. 137°C and was identified as α_1 -sitosterol.

The petroleum ether insoluble fraction, after chromatography on silica gel, gave two flavones. One of them was andrographin, ^I and the other apigenin 4',7-dimethyl ether(I).

The alcoholic mother-liquor, left after separation of the first four crops of crystals, was evaporated to dryness and the residue fractionated with different solvents. The benzene soluble fraction was further fractionated into petroleum ether soluble and insoluble fractions. The petroleum ether soluble fraction furnished further quantities of α_{I-} sitosterol. The petroleum ether insoluble fraction, after crystallisation from ethanol, gave a further quantity of andrographin, while the mother-liquor, on concentration and cooling, yielded a crystalline mass, m.p. 117–180°C. This product on chromatography on silica gel easily gave more α_{I-} sitosterol and mono-O-methylwightin(II) from the first few fractions.

The latter fractions showed mixtures of substances and two of the products seemed to be new. Further investigation on these fraction is in progress.

In a previous report,¹ andrographin m.p. 190–191°C was shown to be a 5-hydroxy-trimethoxyflavone with a molecular formula, $C_{18}H_{16}O_6$. Its degradation, carried out with alcoholic potassium hydroxide, has given *o*-methoxybenzoic acid. Since, no evidence as to the presence of a methoxyl group at the 3position⁴ of the flavone moiety could be obtained, the other two methoxyl groups must be present at either the 6:8, 6:7 or 7:8 positions and andrographin must be either 5-hydroxy-2',6,8-trimethoxyflavone, 5-hydroxy-2',6,7-trimethoxyflavone or 5-hydroxy-2',7,8-trimethoxyflavone (IIIa). Recently, Farkas and Nógrádi⁵ synthesised the last two flavones.



They recorded the melting point of IIIa as 188– 189°C, which agreed very closely with that of andrographin. It thus appeared highly probable that andrographin is 5-hydroxy-2',7,8-trimethoxyflavone (IIIa). In order to confirm this, it was necessary to synthesise IIIa and compare it directly with andrographin. The synthesis was, therefore, carried out following more or less the same procedure as that of Farkas and Nógrádi.⁵ Both the synthetic flavone, and andrographin, and their admixture melted at 190–191°C, thus establishing their identity. They also showed identical behaviour on TLC plates in a variety of solvents. Acetyl derivatives of both the synthetic and the natural flavones and their mixture melted at $155-156^{\circ}$ C. Identity of the two acetates was also established through their identical behaviour on TLC plates.

Panicolin was shown earlier¹ to be a dihydroxydimethoxyflavone having formula $C_{17}H_{14}O_6$, with one hydroxyl group at the 5-position. The position of the other hydroxyl group has now been confirmed by degradative studies. Degradation of panicolin with 30% alcoholic potassium hydroxide readily furnished salicylic acid which proved that the 2'-position of panicolin is substituted with a free hydroxyl group. The two methoxyl groups must be located at the 7 and 8-positions as in andrographin since the free hydroxyl group at the 2'-position in panicolin, when methylated, gave andrographin (IIIa). Thus it was estiblished that panicolin is 2',5-dihydroxy-7,8-dimethoxy flavone (IIIb).

Experimental

Petroleum ether refers to the fraction boiling between $60-80^{\circ}$ C. BDH silica gel (60-120 mesh) and silica gel H were used for column chromatography and TLC respectively.

Extraction of the Roots with Rectified Spirit. Airdried, powdered roots (2.5 kg) of the mature plant, Andrographis paniculata were soaked in rectified spirit (5 1). After standing for 24 hr the greenishvellow extract was run out. This operation was repeated seven times. The combined extracts were distilled under reduced pressure in nitrogen atmosphere. During the distillation, some white solids appeared, which were removed by filtration from time to time and were combined (Fraction A, 4.1 g). The extract was finally concentrated to 11 and kept at 0°C overnight when additional precipitate appeared. After filtration and washing a little cold alcohol. it was obtained as a yellowish green solid (Fraction B; 2.37 g). The mother-liquor and washings were combined together, concentrated to 350 ml and left at 0°C for about a week when it yielded some crystals which were separated as before and obtained as vellowish green needles (Fraction C, 4.70 g). The mother-liquor of fraction C was further concentrated to 200 ml and again kept at 0°C. After about a week some fine needles appeared. They were separated and obtained as pale yellow fine needles (Fraction D, 1.5 g) which melted at 230-240°C. The motherliquor of fraction D was set aside for further examination.

Isolation of Panicolin. Fraction D, m.p. 230–240°C, was crystallised several times from chloroform to give panicolin as pale yellow fluffy needles, m.p. and mixed m.p. 263–246°C, one single spot on TLC plates (R_f 0.05 in benzene); acetate, m.p. 132–133°C. (lit.,^I m.p. 263–264°C for panicolin and m.p. 132–133°C for its acetate).

Isolation of Andrographin and Apigenin 4',7-dimethyl Ether. All the fractions, A, B and C, had indefinite melting points and were contaminated with inorganic materials. They were combined together (11.17g) and extracted repeatedly with boiling chloroform. The chloroform insoluble material (4.8 g) was almost

colourless, and identified as potassium hydrogen phosphate. The combined chloroform extracts were washed with water and dried ($MgSO_4$). After removal of solvent from the chloroform extract the residue was extracted with petroleum ether. The petroleum ether extract, on removal of solvent, vielded a greenish residue (Fraction E; 2.43 g) which was preserved for further examination. The petroleum ether insoluble material (3.84 g) was crystallised from alcohol as vellow needles, m.p. 174-182°C. This material (230 mg) was chromatographed on silica gel (9 g). In all, 22 fractions each of about 20 ml were collected using the following solvent systems: benzene-petroleum ether (3:1; fractions 1-7), benzene-petroleum ether (4:1; fractions 8-10), benzene-petroleum ether (9:1; fractions 11-13), benzene-petroleum ether (9.5:0.5; fractions 14-16), benzene (fractions 17-19) and chloroform (fractions 20-22). All the fractions were examined by TLC on silica gel using benzene-petroleum ether (3:1) as the solvent.

Fractions 13–18 showed one spot on TLC plates and were combined (35 mg). This on crystallisation from methanol gave andrographin as orange yellow hard needles, m.p. and mixed m.p. 190–191°C, highly soluble in chloroform and moderately soluble in alcohol and benzene; acetate, m.p. 155–156°C. (lit.,^I m.p. 190–191°C for andrographin and m.p. 155–157°C for its acetate).

Fractions 20–22 showed one spot on TLC plates and were combined (20 mg). After crystallisation from methanol, this gave apigenin 4',7-dimethyl ether as pale yellow hairy needles, m.p. 170–171°C; acetate, m.p. 193°C. (lit.,⁶ m.p. 170–171°C for apigenin 4,'7dimethyl ether and m.p. 193–194°C for its acetate).

Residues of all other fractions were mixtures of two or more compounds which could not be separated by repeated crystallisation.

Methylation of Panicolin. Panicolin (75 mg), m.p. 263–264°C, was methylated with dimethyl sulphate (0.4 ml) to give andrographin as orange yellow hard needles (70 mg)^I, m.p. and mixed m.p. 190–191°C.

Isolation of α_{I-} Sitosterol and Mono-O-methylwightin. The solvent was removed from the alcoholic mother liquor of the fraction D, and the residue was extracted with petroleum ether. After removal of solvent from the petroleum ether extract, a greenish residue (2.5 g) was obtained. On examination by TLC it appeared to be very similar to the fraction E and was combined with it. The petroleum ether insoluble residue was extracted exhaustively with chloroform. The chloroform insoluble black mass could not be crystallised from any solvent and was rejected. The material, left after removal of the solvent from the chloroform extract, was extracted with benzene. The benzene insoluble material did not furnish any pure compound and was also discarded. The solvent was removed from the benzene extract and the residuc was extracted with petroleum ether again. The petroleum ether extract, after evaporation to dryness, gave a brownish residue (Fraction F; 6 g) which was set aside for further examination. The petroleum ether insoluble fraction of the benzene extract afforded carystalline mass (250 mg) from ethanolic solution

after standing at 0°C for a long period. After crystallisation, once from methanol and again from benzene-petroleum ether, it was obtained as orange yellow needles, m.p. 190–191°C; mixed m.p. with andrographin, 190–191°C. It was identified as andrographin also on the basis of its behaviour on TLC plates.

The ethanolic mother-liquor of the crystalline mass on further concentration and cooling at 0°C for more than a week, furnished a further quantity of yellow crystals (0.9 g), m.p. 117–180°C. Preliminary TLC examination revealed it to be a mixture of several components. This material (0.86 g) was chromatographed on silica gel and 67 fractions each measuring 30 ml were collected using the following solvent systems: petroleum ether (fractions 1–2), benzene– petroleum ether (1:1; fractions 3–10), benzene– petroleum ether (3:1; fractions 11–14), benzene– petroleum ether (5:1; fractions 15–42), chloroform (fractions 43–58), chloroform–ethyl acetate (1:1; fractions 59–63) and ethyl acetate (fractions, 64–67). All the fractions were examined by TLC.

Fractions 3–8, which showed one spot were combined (20 mg), and this yielded α_{I-} sitosterol, m.p. 164°C; acetate, m.p. 137°C (lit., 7 m.p. 164–166°C for α_{I-} sitosterol and m.p. 137°C for its acetate).

Fractions 21–24 which also appeared to consist of one single compound, were combined crystallised once from absolute alcohol and four times from methanol to yield mono-*O*-methylwightin m.p. 190–191°C. (lit.⁸ m.p. 156°C for mono-*O*-methylwightin and m.p. 190–191°C for di-*O*-methylwightin.

All other fractions consisted of mixtures. The combined residues (300 mg) of fractions 48–67 appeared to contain four components on TLC plate (CHCl₃). The substance of R_f 0.24 was shown to be identical with panicolin but the substances of R_f 0.38 and 0.13 were not identical on TLC plates with the flavones and the sterol previously isolated. They seemed to be two new substances. The compound of R_f 0.74 also was not clearly identified with known substances. Attempts for their separation on a silica gel column proved fruitless. Investigation of these substances is in progress.

Examination of Fraction E. The petroleum-ethersoluble fraction (Fraction E; 1.5 g) was chromatographed on silica gel. In all, 28 fractions each of about 30 ml were collected using petroleum etherbenzene (1:1, fractions 1-12), petroleum etherbenzene (1:3; fractions 13-17) and benzene (fractions 18-28). The fractions were examined by TLC. All the fractions consisted of a number of components except fractions 5-12, which showed one major spot on TLC plates. The combined residue (150 mg) of fractions 5-12 was rechromatographed on alumina in petroleum ether-benzene (2:1) and 8 fractions each of about 30 ml were collected. Fractions 6-8 consisted of one single component. They were combined and crystallised from absolute alcohol as white shiny scales (75 mg), m.p. 148-152°C. After three more crystallisations from light petroleum (40-60°C), it furnished α_{I-} situates as white fibrous needles, m.p. and mixed m.p. 164°C.

Examination of Fraction F. The petroleum ether soluble fraction F, after crystallisation from ethanol, afforded an almost colurloess substance (100 mg). It was purified by chromatography first on silica gel with benzene and then on alumina with petroleum ether-benzene (1:1), to give α_{I-} sitosterol.

Degradation of Andrographin. Andrographin 100 mg) was refluxed with 30% alcoholic potassium hydroxide (6 ml) and water (0.5 ml) under nitrogen for 6 hr. After removal of alcohol under reduced pressure, the residue was diluted with water (6 ml) and acidified with concentrated HCl at 0-5°C with stirring. The mixture was saturated with NaCl and extracted with ether (10 ml \times 4). The ethereal solution was extracted with saturated sodium bicarbonate solution (5 ml \times 3), dried (MgSO₄). The solvent distilled leaving off a pale yellow residue (18 mg, fraction A). The bicarbonate extract was acidified to congo-red with sulphuric acid (1:I, v/v) at 0-5°C with stirring. The mixture was saturated with NaCl and extracted with ether (10 ml \times 4). The ethereal extract was washed with ice-cold water (5 ml \times 2) and dried (MgSO₄). Removal of the solvent furnished a crystalline residue (38 mg; fraction B) which was extracted with petroleum ether (10 ml \times 3). The petroleum ether extract, after concentration and cooling, deposited some solids which were crystallised successively from petroleum ether and a mixture of ether and petroleum ether to afford o-methoxybenzoic acid (25 mg) as needles, m.p. and mixed m.p. 102.5-103°C. It produced identical spots with o-methoxybenzoic acid on silica gel TLC plates ($R_f 0.70$) in benzene- methanol-n-butyl-acetate (10:3:3).

The aqueous washing of the fraction B was saturated with NaCl and extracted with ethyl acetate (10 ml \times 3). The extract after drying (MgSO₄) and removal of solvent gave a light brown residue (16 mg) which was found to consist mainly of *o*-methoxybenzoic acid by TLC examination.

The neutral fraction A was found to contain three components by TLC examination. Attempts to separate them by crystallisation was not successful.

Degradation of Panicolin. Panicolin (100 mg) was refluxed under nitrogen with 30% alcoholic potassium hydroxide (6 ml) and water (0.5 ml) for 7 hr. After removal of alcohol under reduced pressure, the residue was diluted with water (4 ml) and acidified to congored at 0–5°C with concentrated hydrochloric acid (52 drops) and extracted with ether (12 ml×4). The ethereal extract was washed with saturated sodium bicarbonate solution (3 ml×4), dried (MgSO₄) and the solvent removed to give a pale yellow residue (41 mg) which consisted of four components as shown by TLC examination, but no pure compound could be obtained.

The sodium bicarbonate washing was acidified to congo-red at $0-5^{\circ}$ C with concentrated hydrochloric acid, saturated with sodium chloride and extracted with ether (12 ml×5). The ethereal extract was dried (MgSO₄) and the solvent removed under reduced pressure to furnish a yellow residue (45 mg) which was taken up into petroleum ether (10 ml×3). The petroleum ether solution, on concentration and cooling, afforded needles (35 mg). This material, after three more crystallisations from the same solvent, gave salicylic acid as colourless shiny needles, m.p. and mixed m.p. with authentic salicylic acid, $161-162^{\circ}$ C. It produced an identical spot (R_f 0.37) with an authentic sample of salicylic acid run side by side on silica gel TLC plates in benzene-methanol-n-butyl acetate (10:3:3).

5-Hydroxy-2'7, 8-trimethoxyflavone. It was synthesised according to the procedure described in the literature⁵ as orangeyellow needles, m.p. 190–191°C; mixed m.p. with andrographin was undepressed.

5-Acetoxy-2'7,8-trimethoxyflavone. 5-Hydroxy-2'7,8trimethoxyflavone (52.8 mg) m.p. 190–191°C, was acetylated according to the method described in the literature^I to give 5-acetoxy-2',7,8-trimethoxyflavone as white fluffy needles (36 mg), m.p. 155–156°C (lit.,⁵ m.p. 156–157°C); mixed m.p. with andrographin acetate was undepressed.

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