Fatty Acid Composition of *Spilanthes paniculata*

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AS a part of our programme to explore the herbal drugs from Indian medicinal plants, we chemically investigated an endemic herb of Tripura, *Spilanthes paniculata* Wall. ex DC[1]. In earlier communication we reported[2] the presence of amino acids in the plant. Now we report the fatty acid composition in the aerial parts of the plant.

Aerial parts of the plant, *S. paniculata* (collected from the area of greater Agartala in September 1985) were air-dried, milled and it (1 kg) was extracted with petrol (b.p. 60–80°) in a Soxhlet for 48 h. The petrol extract was concentrated to a gummy residue (6.5 g). The crude residue (6.0 g) was chromatographed through a silica gel column (60–120 mesh). The latter fraction of petrol eluate and earlier fraction of petrol–benzene (9 : 1) eluate on evaporation of solvent afforded an oily residue (1.5 g). The chemical characteristics of this oil were determined by the usual methods: iodine value (Wij's/30 min), 24.5; saponification equivalent (0.5 *N* alcoholic KOH), 191.5. The oil (1.0 g) was saponified with methanolic 1 *N* KOH (25 ml) for 2 h in an atmosphere of N_2. The solvent MeOH was removed under reduced pressure and the residue diluted with H_2O. The unsaponifiable matter was recovered by extracting the aqueous layer with ether. The ether layer was washed with H_2O, dried with anhydrous Na_2SO_4 and evaporated to get a residue (0.3 g). The mixture of fatty acids was obtained by acidification of the aqueous layer with dilute HCl to pH 2–3 followed by extraction with solvent ether. The ether layer was washed, dried in the usual manner and evaporated to get an oil of fatty acids (0.6 g).

Me-esters of fatty acids were prepared with CH_2N_2 in the usual manner and were purified through silica gel column chromatography. The purified esters mixture was analysed by glc technique on a Pye Unicam 104 gas chromatograph equipped with a FID and a 10% PEGA column. Operating conditions were inj. port temperature 200°, column temperature 190°, FID temperature 210°, and carrier gas N_2 at flow rate 50 ml min^{-1}. The Me-esters were identified[3] by co-glc with authentic samples. The ECL values and relative retention time (with respect to stearic acid) as well as relative abundance (in terms of peak areas) of the Me-esters are given in Table 1.

The presence of unsaturated Me-esters was also confirmed by the catalytic hydrogenation of a part of ester mixture with 10% Pd–C in EtOAc followed by glc analysis under identical condition when the peaks of 10 : 1, 12 : 1, 12 : 2, 14 : 1, 14 : 2, 16 : 1, 18 : 1 and 18 : 2 acids were not observed in the chromatogram and relative intensities of the corresponding saturated acids were increased.

TABLE 1—EQUIVALENT CHAIN LENGTHS (ECL), RELATIVE RETENTION TIME (RRT) AND RELATIVE ABUNDANCE (RA) OF Me-ESTERS

Me-esters of fatty acids	ECL	RRT (18 : 0)	RA %
Capric (10 : 0)	10.00	0.07	2.4
Decamonoenoic (10 : 1)	10.75	0.09	0.8
Lauric (12 : 0)	12.00	0.14	5.0
Dodecamonoenoic (12 : 1)	12.65	0.17	0.6
Dodecadienoic (12 : 2)	13.55	0.23	0.4
Myristic (14 . 0)	14.00	0.27	8.0
Tetradecamonenoic (14 : 1)	14.55	0.32	2.2
Tetradecadienoic (14 : 2)	15.50	0.44	2.2
Pentadecanoic (15 : 0)	15.00	0.38	3.3
Palmitic (16 : 0)	16 00	0.52	55.5
Palmitoleic (16 : 1)	16.50	0.61	0.4
Margaric (17 : 0)	17.00	0.72	0.6
Stearic (18 : 0)	18.00	1.00	18.5
Oleic (18 : 1, n-9)	18.35	0.12	3.8
Linoleic (18 : 2, n-6)	18.95	1.36	0.9
Unidentified	—	—	0 4

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References

1. D. B. DEB, "The Flora of Tripura State", Today & Tomorrow, New Delhi, 1983, Vol. 2, p. 226.
2. B. DINDA and S. GUHA, *J. Indian Chem. Soc.*, Communicated.
3. W. W. CHRISTIE, "Lipid Analysis", 2nd. ed., Pergamon New York, 1982, pp. 63-92.

Amino Acids of *Ajuga macrosperma*

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THE genus Ajuga of the family *Labiatae* comprises of 40 species[1] which are found in temperate regions of the world. Only 5 species[2] are found in India. *Ajuga macrosperma* Wall., an annual hairy herb having flowers in spicate whorls and blue corolla, is found in the upper plain area of greater Agartala. We investigated chemically the whole plant of *A. macrosperma* and detected the presence of twelve amino acids among which six are essential for human system. The identification and relative yields of these amino acids are reported here for the first time.

The whole plants of *A. macrosperma* (collected from Suryamaninagar, Agartala in December, 1986) were air-dried and milled. The milled plant material (300 g) was extracted with petrol (b.p. 60–80°) and rectified spirit successively in a soxhlet for 48 h each. The alcoholic extract was concentrated to a gummy residue (20 1 g). Chromatography[3,4] of a part of the residue (1.3 g) through Amberlite IR 120 (H^+) and elution of the column for neutral and basic amino acids with water and aqueous 4 *N* HCl[5] led to the identification of lysine, arginine, hydroxyproline and valine by co-pc with authentic samples.

Another portion of the crude residue (1.6 g) was chromatographed through Amberlite IRA 400 (OH^-) and elution of the column with water and 2 *N* acetic acid for neutral and acidic amino acids, led to the identification of glycine, proline, methionine, phenylalanine, leucine, valine, tyrosine, aspartic acid and glutamic acid by co-pc with authentic samples. Valine was found also from the column of cation-exchange resin.

All these amino acids were isolated as their ninhydrin complexes by preparative pc[5,6] on Whatmann (3 mm) samples using solvent system n-BuOH–HOAc–H_2O (12 : 3 : 5) as developer. Their relative yields ($\mu g\ g^{-1}$) of the air-dried whole plant based on colorimetric estimation are as follows: proline, 67.9; hydroxyproline, 42 8; methionine, 23.8; tyrosine, 18 4; phenylalanine, 15.3; leucine, 14.2; arginine, 13.5; lysine, 9.2; valine, 9.2; glutamic acid, 2.3; aspartic acid, 1.3; and glycine, 1.3.

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References

1. D. B. Deb, "The Flora of Tripura State", Today & Tomorrow, New Delhi, 1983, Vol. 2, p. 328.
2. J. D. Hooker, "Flora of British India", Bishen Singh Mahendra Pal Singh, Dehra Dun, 1983, Vol. 4, p. 702.
3. E. Lederer and M. Lederer, 'Chromatography", Elsevier, New York, 1957, p. 127.
4. E. Stahl, "Thin Layer Chromatography", 2nd. ed., Toppan, Tokyo, 1969, p. 737.
5. B. Dinda and M. K. Saha, *J. Indian Chem. Soc.*, 1985, 62, 789.
6. D. Abbott and R. S. Andrews, "An Introduction to Chromatography", Houghton Mifflin, Boston, 1965, p. 18.

Chemical Constituents of the Bark and Leaves of *Pterospermum heyneanum* Wall.

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PTEROSPERMUM heyneanum Wall. (Syn. *P. xylocarpum*) is reputed for its medicinal use[1]. Isolation of kaempferol, its 3-*O*-galactoside, kaempferide-7-*O*-glucoside and β-sitosterol were earlier reported[2,3] from the stem cuttings of *P. heyneanum* Wall. In continuation of our work on the plants of Sterculiaceae family[4], we now report the chemical constituents of the bark and leaves of *P. heyneanum*.

The air-dried and powdered bark (1.5 kg) of *P. heyneanum* was extracted successively with n-hexane, chloroform and methanol. The plant waxes were removed from the hexane extract by repeated treatment with methanol and the wax-free residue (6 g) yielded n-triacontanol (100 mg), m p. 84–5°; taraxerone (120 mg), m.p. 240–42°; friedelin (150 mg), m.p. 258–59°; taraxerol (100 mg), m.p. 292–93°; and β-sitosterol (200 mg), m.p. 135–36° on column chromatography. The isolated compounds were identified by their physical and spectral (ir and ^1H nmr) characteristics and direct comparison with the authentic samples.

The residue (4 g) from the chloroform extract on chromatographic resolution over silica gel gave β-sitosterol-3-*O*-β-D-glucoside (250 mg), compound A (25 mg) and aurantiamide acetate[5–7] (60 mg). The residue from the methanol extract yielded only an additional quantity of β-sitosterol glycoside.

Compound A was obtained as a pale yellow oil but it showed homogeneity on tlc (Si-gel, R_f 0.76, C_6H_6–EtOAc, 9 : 1; fluorescent yellow spot when sprayed with methanolic sulphuric acid and heated to 80°). It responded to L–B test for steroids, developed yellow colour with TNM and showed positive Zimmermann colour reaction. Its ir ($CHCl_3$) spectra exhibited bands at 3 450 (OH), 1 720 and 1 680 cm^{-1} (C=O), and the uv spectra (MeOH) showed maxima at 242 (log ε, 4.2) and 273 nm (3.7). Its ^1H nmr spectra showed signals for 4 methyls at δ 0.71–0.98 and two hydrogens of a α,β-unsaturated carbonyl system at δ 5.95 and 6 55 (d, *J* 10 Hz each) in addition to a 1H multiplet at δ 5.22 and a 1H broad singlet at δ 5.68. From the foregoing observations, compound A is considered to be a α,β-unsaturated-ketosterol whose complete characterisation could not be carried out for want of material.

Aurantiamide acetate, $C_{27}H_{28}N_2O_4$ (M^+ 444) was obtained from C_6H_6–EtOAc (9 : 1) eluate and it crystallised from benzene as colourless feathery needles, m.p. 185–86°, $[\alpha]_D$ –26.5°; ν_{max} 3 300, 1 730, 1 660, 1 255, 745, 690 cm^{-1}. The compound was characterised from the results of hydrolysis (6 *N*