Active Anthraquinoids from *Rheum webbianum* Royle

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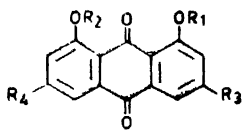
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IN consideration of our interest on *Rheum webbianum* (Polygonaceae) Royle for its commercial use, we examined the roots of the plant and report herein the isolation of 1,8-dihydroxy-3-methyl-, 1,6,8-trihydroxy-3-methyl- and 1,8-dihydroxy-3-hydroxymethyl-anthraquinone.

The roots (700 g) of the plant (collected from Pindari glaciers, 4000 m, in U.P. Kumaon Himalaya) were shade dried, pulverised and soxhlet-extracted with ethanol. The ethanolic extract was concentrated under vacuum and the concentrate was partitioned with $CHCl_3 - H_2O$ (1 : 1, v/v). Both the layers were separated and the chloroform-solubles were concentrated and chromatographed over silica gel G column. On successive elution of the column with n-hexane, n-hexane – benzene and benzene with different proportions, three anthraquinoid-positive fractions (A), (B) and (C) were collected in amorphous forms.

Homogeneity of each fraction was determined by tlc and hplc (Water Associate instrument) using variable wavelength (190 – 750 nm) uv detector.



(A) ; $R_1 = R_2 = R_4 = H$, $R_3 = CH_3$
(B) , $R_1 = R_2 = H$, $R_3 = CH_3$, $R_4 = OH$
(C) , $R_1 = R_2 = R_4 = H$, $R_3 = CH_2OH$

On eluting the silica gel G column with n-hexane – benzene (9 : 1) and on crystallisation of the resulting solid yielded a brilliant yellow coloured compound-A (900 mg), m.p. 192° (M^+ 254) which responded to the usual colour reaction for anthraquinonoid[4] ; the diacetate, m.p. 127° ; λ_{max} (MeOH) 228, 257, 277, 287 and 429 nm ; ν_{max} (KBr) 3 470, 3 080, 1 680 (conjug. C=O) and 1 630 cm^{-1} (chelated C=O) and ^1H and ^{13}C nmr spectral data identified the compound as 1,8-dihydroxy-3-methyl-anthraquinone. Its identity was further ascertained by co-ir and co-hplc with an authentic sample.

Further, on eluting the column with the same solvent n-hexane – benzene (9 : 2), compound B (100 mg), m.p. 250°, was isolated. It formed a trimethyl derivative[4], m p. 205°, and gave characteristic colour reactions[3,4] of anthraquinoids[3,4]. It was identified as 1,6,8-trihydroxy-3-methylanthraquinone by means of spectral data, ms (270, 100%), λ_{max} (MeOH) 222, 226, 252, 289 and 436 nm ; ν_{max} 3 390, 1 675 and 1 630 cm^{-1}, *m/z* 270 (100%) as well as ^1H and ^{13}C nmr spectral data and comparing it with the authentic sample.

The third yellow compound C (80 mg), m.p. 220°, was isolated with the same eluting solvent system n-hexane – benzene (7 : 3). Based on its colour reactions[3,4] and spectral data, ms (M^+ 270, 100%), λ_{max} (MeOH) 225, 254, 276, 287 and 257 sh cm^{-1} ; ν_{max} (KBr) 3 300, 3 050, 1 650 and 1 610 cm^{-1}, ^1H and ^{13}C nmr as well as by comparing the results with an authentic sample, it was identified as 1,8-dihydroxy-3-hydroxymethylanthraquinone.

The spasmolytic activity of the anthraquinoid 1,8-dihydroxy-3-methylanthraquinone is well established[4]. Its presence as a major constituent in the herb seems to be responsible for the spasmolytic properties.

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Amino Acids of *Fleurya interrupta*

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FLEURYA interrupta Gaudich[1,2] (Syn. *F. glomerata* Gaud.) ; (Hindi and Bengali : Lal bichua) belonging to the family *Urticaceae* is the only species recorded in India to be found in Tripura, Khasi hills, Bengal, Bihar, Orissa and South India. The leaves of this plant are reported to be applied locally for carbuncle in Phillippines ; a decoction of the roots is used as a diuretic. We investigated chemically for the first time the aerial parts of this plant. We report herein the isolation, identification and relative occurrence of eleven amino acids, out of which five are essential.

The aerial parts of the young plant (about 3 months old) were collected from the tilla area of Agartala in June 1985. The air-dried milled aerial parts (150 g) were extracted with petrol (b.p. 60 – 80° ; 2 dm^3) and rectified spirit (2 dm^3) sucsessively by percolation. The alcoholic extract

was concentrated to a gummy residue (6.5 g). Chromatography[3,4] of a part of the residue (0.5 g) through Amberlite IR 120(H) and elution of the column for neutral and basic amino acids with distilled water and aqueous 4*N* HCl as described earlier[5], led to the identification of proline, threonine and glycine by co-pc with authentic samples.

Another part of the residue (1.0 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[5] of proline, tyrosine, lysine, alanine, leucine, tryptophan, valine, threonine, aspartic acid and glutamic acid by co-pc with authentic samples. Proline and threonine were also found from the cation resin column.

All these amino acids were isolated from the mixture as their ninhydrin complexes by preparative pc[6] on Whatmann paper (3 mm) using the solvent system n-BuOH – AcOH – H_2O (12 : 3 : 5) as the developing solvent and their relative occurrence (in $\mu g\ g^{-1}$) of the air-dried aerial parts estimated colorimetrically was found to be proline, 19.3; lysine, 2.5; tryptophan, 1.8; threonine, 1.2; glycine, 0.9; alanine, 0.9; tyrosine, 0.5; leucine, 0.4; valine, 0.3; glutamic acid, 0.2 and aspartic acid, 0.1. This appears to be the first report on amino acid composition of the genus, *Fleurya*.

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Spectrophotometric Determination of Fluoride in Water

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FLUORIDES occur in the industrial effluents[1] and also in water flowing over fluoride-containing rocks and minerals. The TLV (threshold limit value) as recommended by the American Conference of Governmental Industrial Hygienists is 3 ppm in drinking water[2]. The toxic effects of excess fluorides are well established[2,3].

The common methods of fluoride analysis are based on the bleaching action of fluorides on the coloured lake formed by various ligands with metal ions, viz. SPADNS and zirconyl chloride[4], eriochrome cyanine R and zirconium, alizarin and cerium[5] etc. The displaced dye differs sufficiently in colour from its metal chelate to permit determination of the fluoride ion concentration as a function of the colour change of the solution. Other spectrophotometric methods make use of liberation of the chelating dye with the addition of fluoride[6,7]. Other methods include tirimetric[8], fluorimetric[9], potentiometric[10] and ion-selective electrodes[11]. All the available methods require close control of pH.

The present method is based on the bleaching action of fluoride on the purple coloured thorium-chromotrope 2B dye complex (λ_{max} 550 nm) and measurement of the decrease in colour intensity at 570 nm, i.e. the wavelength of maximum difference[6]; the pure dye has λ_{max} at 510 nm. The method is simple, rapid and sensitive.

Experimental

All spectral studies were made on a ECIL GS-865 and a Carl Zeiss Specol spectrophotometer using 1 cm matched glass cells.

Standard solution of fluoride (1 mg ml^{-1}) was prepared by dissolving sodium fluoride (A.R.; 0.211 g) in glass-distilled water (100 ml). A 0.002 *M* chromotrope 2B (*p*-nitrobenzeneazochromotropic acid) dye solution was prepared in glass-distilled water. A 0.001 *M* thorium nitrate solution was prepared in glass-distilled water.

Mixed reagent was always prepared fresh by mixing equal volumes of 0.002 *M* chromotrope 2B and 0.001 *M* thorium nitrate to give red-violet complex. Reference solution was prepared by combining the mixed reagent (1 ml) and 0.02 *M* HCl (0.2 ml) and the volume made upto 10 ml with glass-distilled water. Solutions of diverse ions were made by the reported method[12].

Procedure : To the mixed reagent (1 ml) placed in a 10 ml volumetric flask, was added an aliquot of sample solution containing 2.5 – 20 μg of fluoride and the pH maintained at 2.5 – 3.0 by adding 0.02 *M* HCl. Then its volume was made upto the mark with glass-distilled water. After 1 min absorbance was measured at 570 nm against the reference solution.

Results and Discussion

The absorption spectra of chromotrope 2B show that the dye has a maximum absorption at 510 nm. The dye-metal complex has maximum absorption at 550 nm. The maximum difference in the absorption