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PHENOLIC CONSTITUENTS OF *THUJA ORIENTALIS*

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PREVIOUS work on *Thuja orientalis* has revealed the presence of hinokiflavone[1-4], amentoflavone[4], quercetin and myricetin[3] and the occurrence of cupressuflavone was categorically denied[3]. However, the present investigation clearly demonstrates the presence of cupressuflavone which along with amentoflavone and hinokiflavone may serve as a useful taxonomic marker. In addition, the isolation and characterization of apigenin and four flavonol glycosides, besides the reported flavonoids, are being presented. Mono-O-methylamentoflavone has been detected by TLC.

The methanolic extracts of the air-dried and powdered leaves (4 kg) (procured from FRI, Dehra Dun,) were concentrated *in vacuo* to a viscous state and poured in excess of water. The precipitate was successively treated with pet. ether (60–80°), benzene, chloroform and ethyl acetate. The ethyl acetate fraction was concentrated and chromatographed over silica gel column using benzene and benzene-ethyl acetate as eluent. The fractions eluted with benzene-ethyl acetate (9:1) contained apigenin as confirmed by its m p. 346°, m.m p., characteristic shade of its methyl ether in UV light (fluorescent blue) and UV spectral studies. The fractions eluted with benzene-ethylacetate (8:2) contained quercetin and myricetin confirmed by comparison with authentic markers. The fraction eluted with benzene-ethyl acetate (7:3) was found on methylation and TLC examination to be the mixture of cupressuflavone, mono-O-methyl amentoflavone and reported biflavones. The mixture was separated by preparative TLC on silica gel using benzene-pyridine-formic acid (BPF-40:10:2). Cupressuflavone, m p. 359°, its methyl ether m.p. 296° (orange fluorescence in UV light on TLC) and acetate m.p. 250°, was confirmed by comparison of ^{1}H-NMR spectral data of its methyl ether with authentic sample[5,6]. Amentoflavone and hinokiflavone were identified by comparison of chromatographic and spectral data with authentic samples. Mono-methyl ethers of amentoflavone and hinokiflavone could only be detected.

The aqueous phase was successively extracted with ether and ethyl acetate. Ether extract did not yield any substance. The ethyl acetate extract was concentrated under diminished pressure to give a yellowish-brown semi solid mass which on repeated column chromatography on silica gel and polyamide followed by preparative PC yielded the following four compounds:

Compound I: Crystallized from MeOH as yellow needles, m.p. 186°, was analysed for $C_{21}H_{20}O_{11}$. On hydrolysis with 6% HCl it gave quercetin (m p., m.m p. 315°, Co-PC and UV spectral studies) and rhamnose (PC). Hydrolysis of permethylated glycoside yielded quercetin tetramethyl ether which with $AlCl_3$ showed a bathochromic shift of 61 nm (from 358–419 nm) in Band I, thereby suggesting that the rhamnose residue was present at the 3-position[7,8]. The compound was thus identified as quercetin-3-O-rhamnoside (quercitrin)[8].

Compound II: Crystallized from MeOH-C_6H_6 as yellow cubes, m.p. 228°. Acid hydrolysis (6% HCl) of the glycoside afforded kaempferol (m.p., m.m.p. 276°, Co-PC) and glucose (PC). The points of attachment of the sugar moiety in glycoside was deduced from UV spectral shift studies of the glycoside was well as the hydrolysed product of the permethylated glycoside. In the former case no appreciable shift was produced in band II with sodium acetate, while in the latter a bathochromic shift of 9 nm was observed thereby confirming the glycosidation at C-7. Thus the compound was identified as kaempferol-7-O-glucoside[8].

Compound III: It appeared as bright yellow on PC, on hydrolysis with 6% HCl gave quercetin and rhamnose. The UV spectral data were not found comparable with quercitrin. A slight hypsochromic shift in band II was observed with sodium acetate suggesting the glycosidation at 7-position[8]. The compound was thus identified as quercetin-7-O-rhamnoside.

Compound IV: A pale yellow solid, m.p. 195–96 responded to colour tests for flavonol glycoside.

Hydrolysis of the glycoside with 6% HCl for 1 hr yielded myricetin (m.p., m.m.p. 358° and UV spectral studies[8]) and L-rhamnose (PC). The permethylated glycoside on hydrolysis afforded 5,7,3′,4′,5′-pentamethyl ether of myricetin, m.p. 224–25° which with $AlCl_3$ showed a bathochromic shift of 60 nm in band I absorption suggesting the glycosidation at C-3[8]. On acetylation with Ac_2O and pyridine, it gave an acetate, m.p. 140°. The ^{1}H-NMR spectrum ($CDCl_3$, 60 MHz) showed three alcoholic acetoxyls between δ1.95–2.12 and five phenolic acetoxyls between δ2.25–2.45, rhamnosyl methyl doublet at δ0.9 ($J = 6$ Hz), aromatic proton signals at δ6.78 and 7.24 (d, $J = 2.5$ Hz) for H-6 and H-8 respectively and two proton singlet at δ7.7 for H-2′, 6′, thus confirming that the compound was myricetin-monorhamnoside. The rhamnose C-1 proton at δ5.6 appeared as a doublet with $J = 2$ Hz probably due to equatorial-equatorial coupling with H-2″ thereby showing that the rhamnose formed α-linkage to C-3. Thus the glycoside was identified as myricetin-3-O-α-L-rhamnoside[9].

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RESPONSE OF *TOLYPOTHRIX CEYLONICA* TO SODIUM STRESS

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MANY agricultural ecosystems are increasingly becoming salt-affected, thus rendering them inhospitable for good crop production. Such salt-affected soils cover at present an estimated 7 million hectares of potential crop land in India. Singh[1] was the first to suggest the feasibility of reclaiming such soils with cyanobacteria (blue-green algae). While most plants, with the exception of halophytes, fail to flourish on salt-affected soils, certain cyanobacteria have been found to grow successfully in such ecosystems. In general, cyanobacteria show considerable tolerance to salt and osmotic stresses and this property confers on them a reclamative potential in such problem soils[2,3]. However, the physiological basis of salt tolerance in cyanobacteria has not been adequately investigated[4]. The present communication deals with the response of *Tolypothrix ceylonica*, when challenged with increasing concentrations of Na^+ as sodium carbonate.

The algal strain was an isolate from an alkaline soil from Muketshwaram in Andhra Pradesh. The soil was highly alkaline with pH 10.5 and exchangeable sodium percentage of 96.3 (table 1). The alga was grown photoautotrophically in Fogg's nitrogen-free medium[5] (pH 9.5), fortified with different concentrations of Na^+ ranging from 0 to 200 mM at 29 ± 1°C under continuous illumination (2000 lux). Dry weight at the end of 10th day served as the index of growth. Protein was estimated by Lowry's method[6]. Na^+ was estimated by emission flame photometry[7], using an Elico Flame Photometer Model CL 22A. Total extra-

Table 1 *Physico-chemical properties of the soil from which Tolypothrix ceylonica was isolated*

Soil status	Alkali
Sand %	41.0
Silt %	27.0
Clay %	23.5
pH	10.5
E C mmhos/cm	3.98
Exchangeable sodium percentage	96.3
Cation exchange capacity (meq/100 g)	49.37
Organic carbon (%)	1.13
Total nitrogen (%)	0.023
Available P_2O_5 (kg/ha)	1.79