Survey of Anthoxanthins. Part VI. Colouring Matter of* Tamarix troupii. *Constitution of the Aglycone and its Synthesis.*

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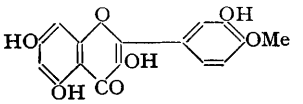
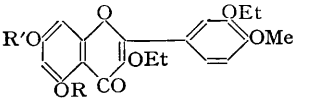
The green leaves of *Tamarix troupii* contain a glycoside, tamarixin, of a new flavonol, whose constitution is established as 3 : 5 : 7 : 3′-tetrahydroxy-4′-methoxyflavone and confirmed by synthesis.

Tamarix troupii is an ornamental plant, parts of which find use in medicine and in tanning. From the dried leaves of *T. gallica* and *T. africana* Perkin and Wood (*J.*, 1898, **73**, 374) isolated ellagic acid and a trace of a yellow flavonol which was considered to be a monomethyl ether of quercetin, different from rhamnetin and *iso*rhamnetin; they, however, were not sure about its purity.

Fresh leaves of *T. troupii*, collected in Delhi, have now been examined: drying brings about adverse changes, and extraction becomes difficult. The colouring matter is a single entity, as shown by paper chromatography. It is a flavonol glycoside, now termed tamarixin. The aglycone, $C_{16}H_{12}O_7$, contains one methoxyl group: on demethylation it yields quercetin, on partial methylation yields quercetin tetramethyl ether (5-hydroxy-3 : 7 : 3′ : 4′-tetramethoxyflavone), and on complete methylation yields quercetin pentamethyl ether. Consequently the methoxyl group is not in position 5. The flavone differs from rhamnetin, *iso*rhamnetin, and quercetin 3-methyl ether (recently prepared in this laboratory; unpublished work); it should therefore be the hitherto unknown 4′-methyl ether (I).

This constitution was confirmed as follows: the aglycone was fully ethylated and the ethyl ether subjected to fission with alcoholic potassium hydroxide, which gave 3-ethoxy-4-methoxybenzoic acid (obtained by the ethylation of *iso*vanillic acid) and ω : 4 : 6-triethoxy-2-hydroxyacetophenone (Perkin, *J.*, 1912, **101**, 329; Row and Seshadri, *Proc. Indian Acad. Sci.*, 1946, **23**, *A*, 140). Ethylation had thus given 3 : 5 : 7 : 3′-tetraethoxy-4′-methoxyflavone (II). This was synthesised by condensing ω-ethoxyphloracetophenone (Row and Seshadri, *loc. cit.*) with the anhydride and the potassium salt of 3-ethoxy-4-methoxybenzoic acid and subjecting the resulting product (III) to further ethylation.

(I) 



(II; R = R′ = Et)

(III; R = R′ = H)

The aglycone (I) has also been synthesised by partial demethylation of quercetin 3 : 4′-dimethyl ether (King, King, and Sellars, *J.*, 1952, 92) as in the analogous synthesis of rhamnazin and kaempferide (Rao and Seshadri, *J.*, 1946, 771; 1947, 122).

Experimental

Extraction.—The fresh leaves (1200 g.) were extracted twice with boiling alcohol, each time for 8 hr. The dark green extract (6 l.) was concentrated under reduced pressure; chlorophyll and waxes separated and were filtered off. The concentrate was repeatedly extracted with light petroleum (b. p. 45—65°), the remaining wax and the green colouring matter being removed. The residual dark brown solution was extracted with ether; the ether extract did not contain any aglycone. An almost colourless solid separated as a fine powder from the aqueous layer. It was filtered off, washed successively with water, alcohol, and ether, and dried (yield, 1·5 g.). The filtrate contained mainly tannins and no anthoxanthins and was discarded. In chromatography (horizontal migration) in which the upper layer of butanol–acetic acid–water (4 : 1 : 5) was used, the solid product gave a single ring (R_F 0·71 at 29—30°). After crystallisation from hot water and from alcohol–water tamarixin was obtained as rectangular prisms, m. p. 315—317° (decomp.). It has not, however, been obtained free from mineral impurities. It is easily soluble in hot water and sparingly soluble in alcohol and other organic solvents.

* Part V, *J. Sci. Ind. Res., India*, 1954, **13**, *B*, 475.

The Aglycone.—Tamarixin (0·5 g.) was refluxed with 7% sulphuric acid (75 c.c.). Though separation of the aglycone started after 15 min., heating was continued for 2 hr. to ensure complete hydrolysis. The mixture was cooled, and the aglycone filtered off and washed with water. More was obtained by extracting the filtrate with ether and evaporating the ether extract, the total yield being 0·31 g. (62%). It has R_F 0·85 in phenol–water (lower layer) at 30°. The *aglycone* crystallised from aqueous alcohol as bright golden-yellow rectangular prisms, m.p. 259—260°. It was insoluble in water, but dissolved readily in alcohol, ether, and acetone. In alcoholic solution it gave an olive-brown colour with ferric chloride, an orange lead salt with neutral and basic lead acetates, and a red colour with magnesium and hydrochloric acid. It gave no colour with zinc and hydrochloric acid (Pew's test), and dissolved in concentrated sulphuric acid to a golden-yellow solution with green fluorescence, and in aqueous sodium hydroxide (1%) and sodium carbonate (5%) to yellow solutions (Found: C, 57·9; H, 4·3; OMe, 9·1. $C_{16}H_{12}O_7,H_2O$ requires C, 57·3; H, 4·2; 1OMe, 9·3%). The *acetyl* derivative crystallised from alcohol as colourless prismatic needles and rods, m. p. 203—204° (Found: C, 59·4; H, 4·3. $C_{24}H_{20}O_{11}$ requires C, 59·5; H, 4·1%).

Methylation of the Aglycone.—(*a*) The aglycone (0·2 g.) in anhydrous acetone (30 c.c.) was refluxed with methyl sulphate (0·22 c.c., 3·2 mols.) and anhydrous potassium carbonate (2 g.) for 10 hr. The methyl ether obtained after removal of the solvent from the filtrate crystallised from ethyl alcohol as pale yellow needles (0·21 g.), m. p. 159—160°. Herzig (*Monatsh.*, 1888, **9**, 541; 1912, **33**, 690) gave the same m. p. for 5-hydroxy-7 : 3 : 3′ : 4′-tetramethoxyflavone. Its alcoholic solution gave a brown colour with ferric chloride.

(*b*) The aglycone (0·2 g.), anhydrous acetone (25 c.c.), excess of methyl sulphate (1 c.c.), and ignited potassium carbonate (3 g.) were refluxed for 50 hr. The product crystallised from alcohol (charcoal) as colourless needles, m. p. 151—152° alone or mixed with quercetin penta-methyl ether.

Demethylation of the Aglycone.—The aglycone (0·5 g.) was heated with acetic anhydride (10 c.c.) and hydriodic acid (10 c.c.) at 145—150° for 2 hr. The cooled mixture was diluted with aqueous sodium hydrogen sulphite, a yellow flocculent precipitate separating, which crystallised from dilute alcohol as yellow needles, m. p. 314—315° (decomp.), alone or mixed with quercetin. With acetic anhydride and pyridine this gave quercetin penta-acetate, needles (from alcohol–benzene), m. p. and mixed m. p. 193—195°.

Ethylation of the Aglycone.—The aglycone (0·3 g.), anhydrous acetone (50 c.c.), ethyl sulphate (excess, 1 c.c.), and ignited potassium carbonate (3 g.) were refluxed for 50 hr. The *tetraethyl ether* crystallised from alcohol as stout prisms, m. p. 136—137° (0·35 g.) (Found: C, 67·3; H, 6·6. $C_{24}H_{28}O_7$ requires C, 67·3; H, 6·5%).

Alkali Fission of the Ethyl Ether.—The ethyl ether (0·3 g.) was heated with alcoholic (8%) potassium hydroxide (30 c.c.). The substance dissolved and soon an orange-red crystalline solid was deposited. After 6 hours' refluxing, the solvent was distilled off, the residue dissolved in water (20 c.c.), and the clear solution acidified and extracted thrice with ether. The ether extract was repeatedly shaken with aqueous sodium hydrogen carbonate. Acidification of the carbonate extract gave colourless *O*-ethyl*iso*vanillic acid, needles, m. p. and mixed m. p. 165—166° (from dilute alcohol).

The remaining ether solution on evaporation gave a small quantity of brown solid, which crystallised from ether–light petroleum as flat needles and plates, m. p. 97—98°. The same m. p. has been recorded for ω : 4 : 6-triethoxy-2-hydroxyacetophenone (Perkin; Row and Seshadri, *locc. cit.*).

For comparison *iso*vanillic acid was obtained as follows: Methyl protocatechuate (18·2 g.) in dry acetone (150 c.c.) was heated with methyl sulphate (10 c.c., 1 mol.) and ignited potassium carbonate (25 g.) for 3 hr. The potassium salts were filtered off and washed with hot acetone. The solvent from the filtrate was then removed by distillation, the residue treated with water, and the colourless solid that separated was filtered off and washed with water. The ether ester so obtained was refluxed with aqueous sodium hydroxide (10%; 150 c.c.) for 2 hr., and then acidified with hydrochloric acid. *iso*Vanillic acid, which separated, crystallised from alcohol as colourless prisms, m. p. 255—257° (13 g.). This acid (16·8 g.) was refluxed with ethyl sulphate (38·5 g.; 2·5 mols.) and potassium carbonate (80 g.) in anhydrous acetone (150 c.c.) for 10 hr. and the resulting ether ester hydrolysed by boiling 10% aqueous sodium hydroxide. *O*-Ethyl-*iso*vanillic acid crystallised from dilute alcohol as colourless needles, m. p. 165—166° (15 g.). Its anhydride was prepared by adding a solution of thionyl chloride (2 c.c.) in ether (25 c.c.) with stirring to a suspension of powdered *O*-ethyl*iso*vanillic acid (9·8 g.) in dry ether (40 c.c.) and pyridine (4 c.c.) cooled in ice–salt. The whole was kept in the refrigerator overnight. Crushed

ice was then added, and the solid was collected, triturated successively with ice-cold dilute hydrochloric acid, dilute aqueous sodium hydrogen carbonate, and water, and dried in a vacuum desiccator. The *anhydride* crystallised from dry benzene as colourless prisms, m. p. 149—150° (7·5 g.) (Found: C, 63·9; H, 6·1. $C_{20}H_{22}O_7$ requires C, 64·2; H, 5·9%).

3 : 3′-*Diethoxy*-5 : 7-*dihydroxy*-4′-*methoxyflavone* (III).—An intimate mixture of ω-ethoxyphloracetophenone (0·4 g.), the anhydride (2·5 g.), and the potassium salt (0·8 g.) of *O*-ethyl-*iso*vanillic acid was heated at 170—180° under reduced pressure for 3 hr. The cooled product was dissolved in ethyl alcohol (20 c.c.), water (2 c.c.) containing potassium hydroxide (2·8 g.) was added, and the solution boiled for 20 min. The solvents were then removed under reduced pressure and water was added. The aqueous solution was extracted once with ether and then saturated with carbon dioxide, and the precipitated *flavone* was collected and crystallised from alcohol, forming prismatic needles, m. p. 183—185° (0·5 g.). Its alcoholic solution gave a reddish-brown colour with ferric chloride (Found, after drying *in vacuo* at 110°: C, 64·4; H, 5·4. $C_{20}H_{20}O_7$ requires C, 64·5; H, 5·4%).

5 : 7 : 3 : 3′-*Tetraethoxy*-4′-*methoxyflavone* (II).—The above diethoxyflavone (0·3 g.) was ethylated with ethyl sulphate (0·4 c.c.) and potassium carbonate (2 g.) in boiling anhydrous acetone (50 c.c.) for 30 hr. The tetraethyl ether crystallised from alcohol as stout prisms, m. p. 136—137° (0·28 g.), gave a negative ferric reaction, and was insoluble in aqueous sodium hydroxide. This was found to be identical with the aglycone tetraethyl ether (mixed m. p.).

5 : 7 : 3 : 3′-*Tetrahydroxy*-4′-*methoxyflavone* (I).—5 : 7 : 3′-Trihydroxy-3 : 4′-dimethoxyflavone (King *et al.*, *loc. cit.*) (0·5 g.) was dissolved in dry nitrobenzene (4 c.c.), treated with a solution of anhydrous aluminium chloride (1 g.) in the same solvent (4 c.c.), and kept in a boiling-water bath for 1½ hr. The mixture was then cooled and excess of light petroleum added. The brown precipitate was filtered off and washed with light petroleum. It was then treated with dilute hydrochloric acid, the mixture was heated nearly to boiling and filtered, and the solid residue washed with water. The product (0·4 g.) crystallised from dilute alcohol as rectangular prisms m. p. and mixed m. p. 259—260°. The acetyl derivative crystallised from alcohol as prismatic needles and rods, m. p. and mixed m. p. 203—204°.

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