THE STRUCTURE OF TAMARIXIN

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Seshadri and Gupta [1], who discovered tamarixin (Ia) in the Indian plant Tamarix troupii, considered it to be a glycoside of 4'-O-methylquercetin [tamarixetin (Ib)]; however, they gave no proof of the presence of a sugar in the molecule of this flavonoid. Meanwhile, Hattori [2] in a review of flavone glycosides has stated that tamarixin is the 3-O-glucoside of tamarixetin.

We have investigated the epigeal parts of Tamarix laxa Willd. (collected by P. S. Massagetov's expedition in Kazakhstan in the summer of 1961) and have isolated from them a substance with mp 316° C which on hyrolysis with acid gave a flavone of the composition $C_{16}H_{12}O_7$. In spite of numerous recrystallizations, its melting point could not be raised above 252° C, while according to the Indian workers tamarixetin melts at 259°-260° C. The flavone that we obtained gave a tetraacetate melting at 202° C, which agreed with the melting point of tamarixetin tetraacetate. We have previously had occasion to observe [3] that flavones sometimes cannot be separated from impurities by recrystallizations; in such cases, this is achieved by converting the flavones to the acetates with subsequent saponification of the latter. Consequently, we hydrolyzed the acetate by heating it with hydrochloric acid, but this led to the initial flavone with the original melting point. In order to eliminate any doubt relating to the nature of the aglycone, we ethylated it and obtained a product the composition of which corresponded to that of an O-methyltetra-O-ethylquercetin and the melting point of which was again lower than that given for this derivative by Seshadri and Gupta [1]. Decomposition of the quercetin derivative with alcoholic caustic potash led to O-ethylisovanillic acid and ω,4,6-triethoxy-2-hydroxyacetophenone, as with the Indian workers. The solution obtained after the hydrolysis of tamarixin with hydrochloric acid and separation of the precipitated "aglycone" did not reduce Fehling's solution, but contained sulfate and potassium ions. It was naturally assumed that tamarixin, like persicarin or the rhamnazin derivative isolated by Hörhammer [4] is an ester of tamarixetin and potassium hydrogen sulfate, which was confirmed by a quantitative determination of the sulfuric acid formed in the hydrolysis of tamarixin. This explains why Seshadri and Gupta could not obtain tamarixin free from mineral impurities in spite of a number of recrystallizations.

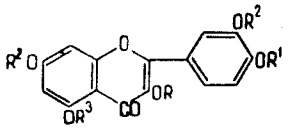
Tamarixin does not give a yellow coloration with zirconium oxychloride and citric acid [4]. Consequently, the potassium hydrogen sulfate esterifies the hydroxyl group in position 3 of tamarixetin. We determined the position of the potassium hydrogen sulfate by the general method of methylating the free hydroxyl groups of the flavone with diazomethane, hydrolysis of the product, and identification of the tetra-O-methyl derivative of quercetin so formed. The experiments showed that the methylation of tamarixin takes place slowly because of its poor solubility in organic solvents, and cannot be brought to completion even on prolonged standing with a solution of diazomethane. The methylation product remained in the form of a solid from which, after hydrolysis with acid and crystallization from methanol we obtained two substances. The first (mp 183°-184° C) was 7,3',4'-tri-O-methylquercetin (Ic), which has been described by Kuhn [6], and the second had a melting point of 170°-172° C and a composition close to that of a tri-O-methylquercetin; the formation of this product has been recorded by Attree and Perkin [7] by the incomplete methylation of xanthorhamnin. They considered this substance to be a mixture of 7,3',4'-trimethylquercetin with a small amount of 5,7,3',4'-tetramethylquercetin (Id) that they could not separate by crystallization; however, they were unable to prove this. We were able to confirm the assumption of the English workers, since after acetylation of the substance with mp 170°-172° C we obtained a mixture of two acetates which was readily separated into 3,5-diacetyl-7,3',4'-trimethylquercetin (Ie) and 3-acetyl-5,7,3',4'-tetramethylquercetin (If).

The UV absorption spectrum of tamarixin (taken by E. M. Peresleni on a SF-4 spectrophotometer) shows a considerable hypsochromic displacement of the first absorption maximum as compared with the spectrum of tamarixetin. Such a displacement is found in flavonols when the hydroxyl group in position 3 is substituted [8]. The position of the first maximum in the spectra of the tri-O-methyl derivative (Ic) and the tetra-O-methyl derivative of quercetin (Id) corresponds to the presence in them of a free hydroxyl group in position 3.

Substance	λ_{max}, mμ (log ε)			
Quercetin	371(4.32)		257(4.31)	
Tamarixetin	370(4.31)	298a*	255(4.30)	
Tamarixin	346(4.20)	290a	265(4.26)	255(4.24)
Penta-O-methylquercetin	342(4.34)	266a	250(4.34)	
5,7,3',4'-Tetramethylquercetin	365(4.38)		252(4.37)	
7,3',4'-Trimethylquercetin	370(4.35)	302a	255(4.34)	

*a - point of inflection; the determinations were performed in alcohol

Thus, tamarixin is 4'-methylquercetin esterified with potassium hydrogen sulfate in position 3.



1a: R = SO_3K, $R^1 = CH_3$, $R^2 = R^3 = H$;
1b: R = $R^2 = R^3 = H$, $R^1 = CH_3$;
1c: R = $R^3 = H$, $R^1 = R^2 = CH_3$;
1d: R = H, $R^1 = R^2 = R^3 = CH_3$;
1e: R = $R^3 = OCCH_3$, $R^1 = R^2 = CH_3$;
1f: R = $OCCH_3$, $R^1 = R^2 = R^3 = CH_3$;
1g: R = $R^2 = R^3 = C_2H_5$, $R^1 = CH_3$.

Experimental

Isolation of tamarixin. The comminuted green part of the plant (320 g) was extracted in a Soxhlet apparatus with 1200 ml of methanol for 18-20 hr. The methanol was distilled off from the extract until the residue foamed, and then 75 ml of water and 75 ml of chloroform were added and, after shaking, the mixture was left in the refrigerator. A crystalline precipitate was deposited, which was washed with chloroform and water. The yield of dry matter was 2.7 g. Recrystallization from 100 parts of hot 50% methanol gave 1.25 g of tamarixin (Ia) in the form of pale yellow rhombic plates with mp 316°-317° C. The literature data gives mp 315°-317° C [1]. For analysis (here and below), the substances were dried at 100° C and 2 mm.

Hydrolysis of tamarixin. A mixture of 0.6534 g of tamarixin and 60 ml of 1 N hydrochloric acid was heated on a boiling water bath for 2 hr; the substance rapidly went into solution, and after 7-10 min a bright yellow precipitate began to separate. After the mixture had been cooled, the precipitate was transferred quantitatively to a glass filter, and it was washed with water and dried in a vacuum desiccator over sulfuric acid. This gave 0.4683 g of tamarixetin. The sulfuric acid in the filtrate was precipitated with barium chloride, to give 0.3436 g of barium sulfate.

Found, %: C 43.79; H 2.89; S 7.22; CH_3O 7.14; tamarixetin 71.67%. Calculated for $C_{16}H_{11}O_{10}SK \cdot 0.5\ H_2O$, %: C 43.35; H 2.72; S 7.23; CH_3O 7.00; tamarixetin 71.34%.

When the tamarixetin was recrystallized from 75% methanol, small yellow needles with mp 251°-252° C were formed; the literature gives mp 259°-260° C [1].

Found, %: C 60.36; H 3.95. Calculated for $C_{16}H_{12}O_7$, %: C 60.75; H 3.82.

The tamarixetin was acetylated by boiling with acetic anhydride and sodium acetate. After the usual working up, fine colorless needles of the tetraacetate with mp 201°-202° C (from methanol) deposited; the literature gives mp 203°-204° C [1].

Found, %: C 59.33; H 4.16. Calculated for $C_{24}H_{20}O_{11}$, %: C 59.51; H 4.16.

For its deacetylation, the tetraacetate (0.3 g) was heated with 10 ml of hydrochloric acid (sp. gr. 1.19) on a boiling water bath for 1 hr. After dilution of the solution with water, a bright yellow precipitate was deposited which had mp 251°-252° C (from 80% methanol).

Demethylation of tamarixetin. A mixture of 0.5 g of tamarixetin, 10 ml of hydriodic acid (sp. gr. 1.40) and 10 ml of acetic anhydride was boiled for 2 hr; after cooling, it was diluted with 100 ml of 5% sodium sulfite solution, and the yellow precipitate was recrystallized from 80% methanol and acetylated as described above. The colorless needles with mp 192°-193° C (from methanol) gave no depression of the melting point in admixture with quercetin pentaacetate.

3,5,7,3'-Tetraethyl-4'-methylquercetin (Ig). A mixture of 0.95 g of tamarixetin, 4 ml of diethyl sulfate, 10 g of calcined potassium carbonate, and 100 ml of acetone was boiled for 51 hr. After the separation of the salts and the distillation of the acetone, the residue was treated with 10% ammonia to decompose the excess of diethyl sulfate. The residue was recrystallized from 60% methanol, giving clusters of colorless needles with mp 128°-129° C; the literature gives mp 136°-137° C [1].

Found, %: C 67.58; H 6.38. Calculated for $C_{24}H_{28}O_7$, %: C 67.28; H 6.59.

A mixture of 0.3 g of the substance, 2 g of caustic potash, and 25 ml of absolute alcohol was boiled for 6 hr and then the decomposition products were separated by the usual method. A crystalline acid with mp 164° C (from 60% methanol) which gave no depression of the melting point in admixture with an authentic sample of O-ethylisovanillic acid was isolated from the acid fraction; this fixes the position of the 4'-methoxyl group.

7,3',4'-Trimethylquercetin, its 3,5-diacetate (Id) and 3-O-acetyl-5,7,3',4'-tetramethylquercetin (If). A solution of diazomethane from 3 g of nitrosomethylurea in 30 ml of ether was added to a suspension of 0.9 g of tamarixin in

20 ml of methanol. The red coloration that appeared disappeared again fairly rapidly. After one day and three days, similar amounts of diazomethane solution were added. Distillation of the ether showed that the solution contained a large excess of diazomethane. The residue was hydrolyzed by heating on a boiling water bath with 40 ml of 1 N hydrochloric acid for 2 hr. The yellow precipitate (0.65 g) was recrystallized from 65 ml of boiling methanol with activated carbon. Yield 0.4 g, mp 167° C. When recrystallization was attempted, a considerable part of the substance did not dissolve in 80 ml of boiling methanol and was filtered off, mp 186°–187° C. Literature data for 7,3',4'-trimethylquercetin (Ic) gives mp 183°–184° C [6].

Found, %: C 62.76; H 4.70; CH_3O 26.38. Calculated for $C_{18}H_{16}O_7$, %: C 62.77; H 4.68; CH_3O 27.03.

The substances was acetylated as described above; the acetyl derivative had mp 193°–194° C (from methanol). Literature data for 3,5-diacetyl-7,3',4'-trimethylquercetin (Ie) gives mp 187°–188° C [6].

Found, %: C 61.64; H 4.71. Calculated for $C_{22}H_{20}O_9$, %: C 61.68; H 4.71.

On cooling, the filtrate, after the separation of the tri-O-methylquercetin, deposited yellow needles with mp 170°–172° C. A mixture of 0.2 g of this substance, 2 ml of pyridine, and 5 ml of acetic anhydride was heated until the crystals dissolved and was then left for a day at 20° C. Then the solution was evaporated under vacuum. The resid resin was dissolved in acetone, the solution was boiled with carbon, the acetone was distilled off, and 3 ml of ether w added to the residual concentrated solution. A pronounced turbidity formed, which disappeared on heating, and on co ing aggregates of fine needles with mp 161°–162° C (from methanol) were produced. Literature data for 3-acetyl-5,7 3',4'-tetramethylquercetin (If) gives mp 160°–163° C [9].

Found, %: C 62.01; H 5.31. Calculated for $C_{21}H_{20}O_8 \cdot 0.5\ CH_3OH$, %: C 62.00; H 5.33.

After the mother liquor had been treated with 5 ml of ether and allowed to stand, we isolated from it lustrous crystals with mp 192°–193° C (from methanol) which gave no depression of the melting point in admixture with the di acetate of 7,3',4'-trimethylquercetin (Ie) described above, and had the same composition.

5,7,3',4'-Tetramethylquercetin (Id) and its acetate (If). For a direct comparison of the derivatives obtained, methylated hyperin, quercetin 3-D-galactoside, isolated from the leaves of Hypericum perforatum L. (common St. John's wort) by a known method [10]. The hyperin, with mp 235°–237° C (from alcohol), was methylated with diazomethane as described above for tamarixin. After hydrolysis of the methylated glycoside and recrystallization of the aglycone from methanol, we obtained 5,7,3',4'-tetramethylquercetin (Id) with mp 188°–189° C. Literature data gives mp 189°–190° C [10].

Found, %: C 63.52; H 5.01. Calculated for $C_{19}H_{18}O_7$, %: C 63.67; H 5.06.

The substance was acetylated with acetic anhydride in pyridine as described above. An acetate with mp 160°–161° C (from methanol) deposited, which gave no depression of the melting point in admixture with the acetate of tetra-O-methylquercetin (If) from tamarixin.

The elementary analyses were performed in the microanalytical laboratory under the direction of V.V. Kolpakc

Summary

Tamarixin, isolated from the central Asiatic plant Tamarix laxa Willd., is 4'-methylquercetin esterified with potassium hydrogen sulfate in position 3.

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