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DETERMINATION OF CONSTITUTION AND SYNTHESIS OF A NEW FLAVONE FROM *CISTUS MONSPELIENSIS* L.*

G. Berti, O. Livi, D. Segnini and I. Cavero
Institutes of Organic and Pharmaceutical Chemistry, University of Pisa, Italy

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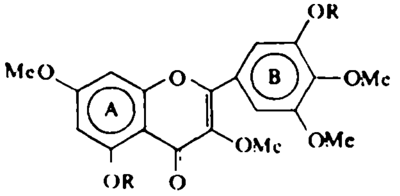
Abstract—A new flavone, isolated from *Cistus monspeliensis*, has been identified as myricetin-3,7,3',4'-tetramethyl ether [I], supported by UV, IR and NMR data and degradation experiments. The new compound has also been prepared by an Allan-Robinson type synthesis.

With the exception of *Cistus labdaniferus*,[1] the genus *Cistus* (*Cistaceae*) has not been investigated to any extent. During a chemical study of the diterpenes present in *Cistus monspeliensis*, a shrub widely distributed in Mediterranean regions, a new flavone was isolated and has now been characterized. This compound is readily obtained pure in 0·1% yield (based on the dry plant) from the phenolic portion of a petroleum ether extract. It has m.p. 149–151° and analysed for $C_{19}H_{18}O_8$. The IR and UV visible spectra, as well as the usual color tests indicated that the new compound was a flavone derivative. As it formed diacetyl and dibenzoyl derivatives and showed four methoxy groups in a Zeisel determination, it must be a dihydroxy-tetramethoxyflavone.

Table 1. NMR spectral data



Compound	R	Types of protons	τ (Number of protons)	Shape of peaks (J)
I	H—	MeO—	6·23(6); 6·17(3); 6·12(3)	Singlets
		5-OH	−2·2(1)	Singlet
		Ring A	3·86(1); 3·72(1)	AB quartet (2c/s)
		Ring B	2·90(2)	unresolved
II	Ac—	MeO—	6·16(3); 6·08(3); 6·03(3); 6·02(3)	Singlets
		Ac—	7·60(3); 7·51(3)	Singlets
		Ring A	3·42(1); 3·20(1)	AB quartet (2c/s)
		Ring B	2·60(1); 2·40(1)	AB quartet (2c/s)
III	PhCO—	MeO—	6·19(3); 6·09(3); 6·07(3); 6·01(3)	Singlets
		Ring A	3·30(1); 3·16(1)	AB quartet (2c/s)
		PhCO— and ring B	2·61–1·60(7)	Multiplets*
V	Me—	MeO—	6·14(3); 6·09(3); 6·04(12)	Singlets
		Ring A	3·70(1); 3·54(1)	AB quartet (2c/s)
		Ring B	2·65(2)	Singlet

* The signals of the benzoyl protons overlap those of the ring B protons.

The NMR spectral data (Table 1) confirmed the presence of four MeO groups

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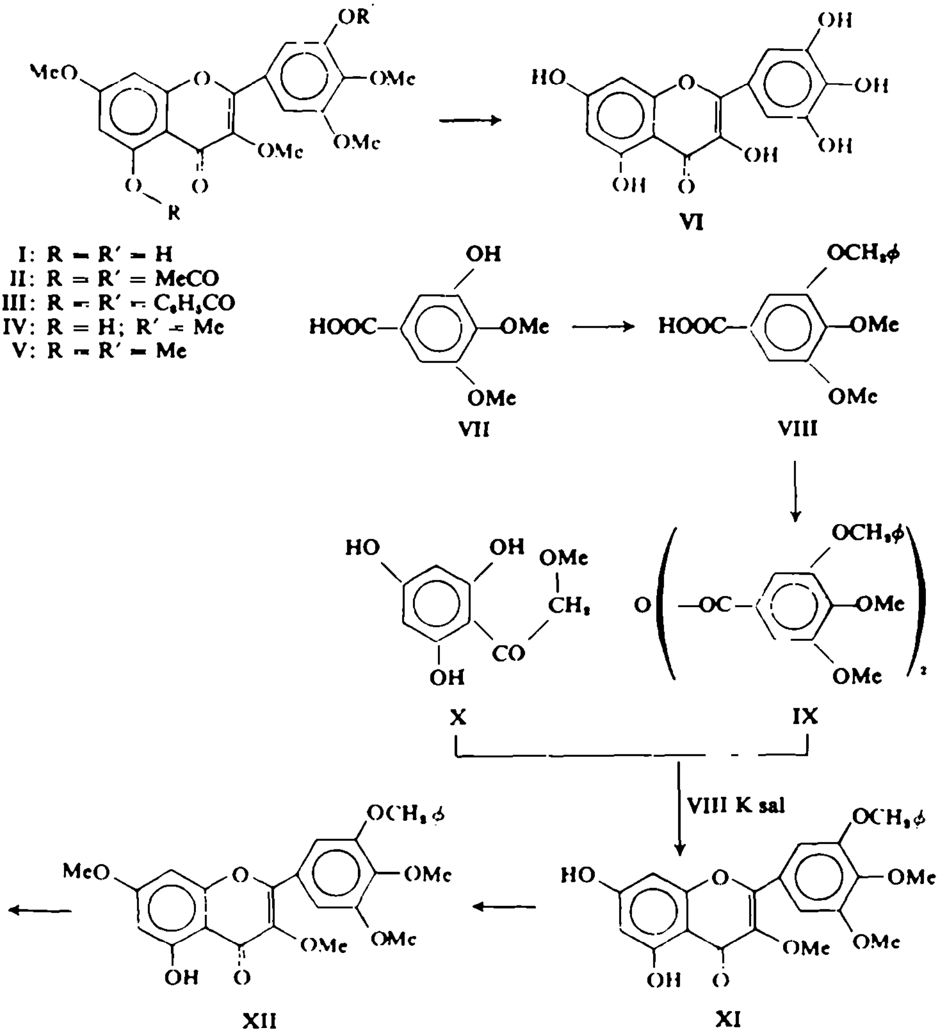
[*] Dedicated to Prof. Remo de Fazi, on his 75th birthday.

[1] R. Hegnauer, *Chemotaxonomie der Planzen* Vol. III; p. 429. Birkhäuser Verlag, Basel (1964).

and in addition, a one-proton singlet at $-2\cdot2\ \tau$ indicated a strongly hydrogen-bonded OH group, which could be placed with fair certainty in the 5 position.[2] The aromatic protons appeared in two groups of signals: a two-proton AB quartet centered at $3\cdot79\ \tau$ (J 2 c/s) and an unresolved two-proton peak at $2\cdot90\ \tau$. On the basis of the data in the literature,[2] the signals at higher field can be attributed to the protons on ring A, while the value of the coupling constant indicates that they are *meta* to each other: therefore ring A must be 5,7-disubstituted.



I: R = R' = H
II: R = R' = MeCO
III: R = R' = C_6H_5CO
IV: R = H; R' = Me
V: R = R' = Me

The NMR spectrum of the diacetyl derivative indicated besides the AB quartet of the two ring A protons (now centered at $3\cdot31\tau$), two protons of ring B as a well-resolved AB quartet centered at $2\cdot50\ \tau$ (J 2 c/s). Therefore, also in ring B the two

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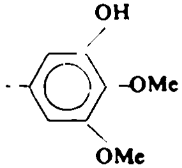
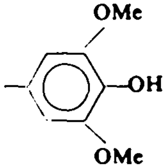
[2a] J. Massicot and J.-P. Marthe, *Bull. Soc. Chim. Fr* 1962 (1962);
[b] T. J. Batterham and R. J. Highet, *Austral. J. Chem.* 17, 428 (1964);
[c] C. A. Henrick and P. R. Jefferies, *Ibid.* 17, 934 (1964).

aromatic protons must be *meta* to each other. This pointed strongly to a myricetin type of structure, i.e. substitution in the 3′, 4′ and 5′ positions, since the other possibilities, namely 2′, 3′, 5′ and 2′, 4′, 6′ substitution have not been encountered in natural flavones[3] and also are unlikely from a biogenetic point of view.[4] Furthermore, if it was assumed that the compound was a myricetin derivative, the fact that acetylation produced such a marked change on the ring B aromatic protons could be explained only by placing one of the two free OH groups on this ring: this left the two possibilities XIII and XIV; however, only the former could explain the NMR data as only an acetoxy group placed asymmetrically with respect to the two aryl protons could justify their non-equivalence, and therefore the appearance of the AB quartet.

In conclusion the NMR data pointed strongly to the formulation of the new flavone



XIII



XIV

as I. This was confirmed as follows. Demethylation with hydrogen iodide gave a hexahydroxyflavone, which corresponded in all its properties to myricetin (VI). Methylation of I with diazomethane at 0° for 2 hr produced a monomethyl derivative (IV), which was almost insoluble in alkali, while the second OH group was methylated only under more drastic conditions (diazomethane at room temp for 20 hr or treatment with dimethyl sulphate and potassium carbonate for 26 hr); these facts confirm the presence of a free OH group in 5 position, as it is well known that such a group is less acidic and reactive than hydroxyls in any other position of the flavone system;[5] furthermore the physical constants of the mono- and dimethyl derivatives of the new flavone were in accordance with those reported for myricetin 3, 7, 3′, 4′, 5′-pentamethyl and hexamethyl ether.

Alkaline degradation of I gave, as expected, 3-hydroxy-4,5-dimethoxybenzoic acid, thus confirming a substitution pattern of type XIII for ring B.

A final proof of constitution I was given through the synthesis by the Allan-Robinson method.[6] 3-Hydroxy-4,5-dimethoxybenzoic acid (VII) was transformed into the 3-benzyl ether VIII, by benzylation of its methyl ester, followed by hydrolysis. Reaction of a mixture of the potassium salt of VIII and of its anhydride IX (prepared from VIII with thionyl chloride in pyridine) with 2,4,6-trihydroxy-ω-methoxyacetophenone gave the flavone derivative XI, which was monomethylated with dimethyl sulphate. As said above, a OH group in the 7 position is methylated more easily than one in the 5 position;[5] therefore the monomethylation product must be XII. Debenzylation of the latter compound with hydrogen chloride in acetic acid yielded a product I, which was identical with the natural compound.

While myricetin is a very widely diffused flavonoid, only very few reports can be

[3] F. M. Dean, *Naturally Occurring Oxygen Ring Compounds* p. 318. Butterworth, London (1963).

[4] H. Grisebach, in T. W. Goodwin, Editor, *Chemistry and Biochemistry of Plant Pigments* p. 279. Academic Press, London (1965).

[5] Ref. 3, p. 284.

[6] J. Allan and R. Robinson, *J. Chem. Soc.* 2334 (1926).

found on the isolation of its methyl ethers. The 5-methyl ether was obtained from rhododendron blooms,[7] the 3,7,4′-trimethyl ether from *Ricinocarpus stylosus*,[2c] and the 3,7,3′,4′,5′-pentamethyl ether from *Combretum quadrangulare*.[8]

EXPERIMENTAL

M.ps were taken with a Kofler apparatus. IR spectra were recorded with a Perkin–Elmer Infracord Mod. 137 spectrophotometer on mineral oil mulls, UV spectra in EtOH soln with a Beckman DU. NMR spectra were obtained in $CDCl_3$ soln with a Varian HR-60 spectrometer [TMS as internal standard]. Elemental analyses were performed by A. Bernhardt, Mülheim (Germany).

Isolation and characterization of I

Cistus monspeliensis was collected on Giglio island in September 1965 and the leaves were subjected to steam-distillation and removal of a small amount of volatile oil. The residue after distillation was pressed, dried at 40° and ground. The powder (2,870 g) was extracted for 53 hr with 13 litres of pet. ether (b.p. 40–60°).

Concentration of the extract to 1·5 litres resulted in the separation of a dark viscous oil, from which the supernatant soln was decanted. The oil was dissolved in 700 ml Chf and the soln was washed with 500 ml 6% $NaHCO_3$aq; acidification yielded a solid diterpene acid, which is being investigated. The washing was continued with four 250-ml portions of 2N NaOH. Acidification of the latter washings produced a semi-solid ppt, which after several crystallizations from benzene yielded 3·2 g I, in a solvated form containing benzene, m.p. 145–150°. The solvent-free product, m.p. 149–151°, was obtained by sublimation at 200°/1 mm. (Found: C, 61·32; H, 5·09; OMe, 32·35. $C_{19}H_{18}O_8$ requires: C, 60·96; H, 4·85; 4 OMe, 33·16%.) λ_{max}(log ε) 265 (4·26), 306 (4·12, infl.) and 348 mμ (4·25); λ_{OH} 2·91, λ_{CO} 6·07μ. NMR spectrum, see Table 1.

I is soluble in 2N NaOH and hot 2N HCl, and gives a positive response in the following flavone reactions:[9] green color with $FeCl_3$ in EtOH; immediate red color with Mg and conc. HCl; yellow color with Na–Hg, which turns to magenta after acidification; yellow soln in NaOHaq, which does not change after bubbling air through it (no OH in 3); red dye with phenyldiazonium chloride; positive Wilson boric acid test.

Diacetate of I [II]. The flavone I (55 mg) was refluxed 90 min with 2·5 ml Ac_2O and 2·5 ml pyridine, and the soln was poured into water to give 65 mg white solid, which crystallized from EtOH as plates, m.p. 188–190° (dec); λ_{CO} 5·68, 6·13μ; NMR spectrum, see Table 1. (Found: C, 60·22; H, 4·91. $C_{23}H_{22}O_{10}$ requires: C, 60·26; H, 4·84%.)

Dibenzoate of I [III]. The flavone I (55 mg) was refluxed 90 min with 0·12 ml benzoyl chloride and 2 ml pyridine. After one night at room temp the soln was poured on ice: the white ppt (85 mg) crystallized from acetone in plates, m.p. 248–250° (dec); λ_{CO} 5·73, 6·16 μ; NMR spectrum, see Table 1. (Found: C, 68·50; H, 4·67. $C_{33}H_{26}O_{10}$ requires: C, 68·03; H, 4·50%).

*5-Hydroxy-*3,7,3′,4′,5′*-pentamethoxyflavone* (IV). A soln of 0·1 g I (0·27 mmole) in 20 ml MeOH was cooled at 0° treated dropwise with 7 ml of a 1·5% soln of diazomethane and left 2 hr at 0°. Excess diazomethane was decomposed with AcOH and the solvent was evaporated. The residue was dissolved in 25 ml Chf and washed with three 10-ml portions of 3% KOHaq. Acidification of the alkaline washings led to the recovery of 80 mg I, while evaporation of the Chf layer gave 20 mg of IV, which crystallized from MeOH in pale yellow needles, m.p. 140–142°; λ_{max} 268 and 345 mμ (EtOH), 289 and 388 mμ (0·1N ethanolic NaOH), in accordance with the literature data for myricetin pentamethyl ether.[2c]

3,5,7,3′,4′,5′*-Hexamethoxyflavone* (V). (a) A soln of 0·1 g I in 10 ml ether and 10 ml MeOH was treated with 15 ml of a 1·5% soln of diazomethane in ether and left at room temp for 20 hr, then treated with a few drops AcOH and concentrated to 2 ml. A colorless solid (80 mg) separated and was recrystallized from EtOH to give V, needles, m.p. 154–155°; lit.[2c,10] m.p. 155–156°; λ_{CO} 6·14 μ. (Found: C, 62·59; H, 5·68. Calc. for $C_{21}H_{22}O_8$: C, 62·68; H, 5·51%.)

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[7] K. Egger, *Z. Naturforsch.* **17b**, 489 (1962).

[8] S. Mongkolsuk, F. M. Dean and L. E. Houghton, *J. Chem. Soc.* Sec. C, 125 (1966).

[9] K. Venkataraman, *Fortsch. Chem. org. Naturstoffe* **17**, 1 (1959).

[10] T. R. Seshadri and V. Venkateswarlu, *Proc. Indian Acad. Sci.* **23A**, 296 (1946); *Chem. Abstr.* **40**, 6447 (1946).

(b) A soln of 0·13 g I in 8 ml dry acetone was treated with 0·5 g anhyd K_2CO_3 and 1 ml Me_2SO_4 and refluxed 26 hr. The solvent was evaporated and the residue taken up in water and extracted twice with Chf. The extract was washed with 2N Na_2CO_3 and dried over $MgSO_4$. Evaporation and crystallization of the residue (0·13 g) from EtOH gave V, m.p. 154–155°, whose IR spectrum was identical to that of the product prepared as described under (a).

Demethylation of I. A mixture of 0·13 g I, 4 ml of 57% HI and 1·4 ml Ac_2O was heated 2 hr at 120° and 1 hr at reflux temp. The red hydroiodide was decomposed by leaving it in aqueous suspension overnight: 0·1 g of a yellow solid was obtained. It had no definite m.p., but decomposed slowly above 270°. When its aqueous suspension was treated with a few drops of 0·1N KOH it gave immediately an emerald green color, which slowly changed to blue and then to violet: such a behaviour is typical of myricetin (VI),[11] and this was confirmed by the preparation of the hexacetyl derivative, m.p. 212–213°; lit.[12] for hexacetylmyricetin, m.p. 214–215° (corr).

Alkaline degradation of I. A soln of 0·2 g I in 5 ml EtOH and 15 ml 50% KOH was refluxed 20 hr under N. The EtOH was distilled off under reduced press, 10 ml H_2O was added and CO_2 was bubbled through the soln for 3 hr. The ppt was eliminated by filtration and the filtrate extracted with ether, acidified and extracted again with ether. The dried ($MgSO_4$) ether extract was evaporated to give 80 mg of a solid, which crystallized from water in colorless needles of VII, m.p. 194–195°; lit.,[13] m.p. 193–194°. Its IR spectrum was identical with that of a sample of VII, which had been obtained synthetically.

Synthesis of I

3-*Benzyloxy*-4,5-*dimethoxybenzoic acid* (VIII). A soln of 4·65 g (22 mmole) of VII methyl ester[13,14] in 15 ml MeOH was treated with 2·49 g (19·7 mmole) benzyl chloride and 10 ml of a 12·5% soln of KOH in MeOH (22·3 mmole), refluxed until it became neutral (3 hr), ¾ of the solvent was distilled off, water was added, and the ppt extracted with ether. The ether extract was washed with 2N NaOH (0·8 g of VII was thus recovered), dried ($MgSO_4$) and evaporated. The residue (4·6 g, m.p. 68–70°) of VIII methyl ester was boiled until it dissolved completely with 8 ml 10% NaOHaq, the soln was diluted with 20 ml water and extracted with ether. The aqueous layer was acidified with conc. HCl and the ppt was crystallized from EtOH to give 4 g VIII, m.p. 172–174°; lit.[15] m.p. 173–174°.

3-*Benzyloxy*-4,5-*dimethoxybenzoic anhydride* (IX). A suspension of 4·5 g finely powdered VIII in 15 ml dry ether and 1·7 ml pyridine was treated dropwise with a soln of 0·8 ml $SOCl_2$ in 4 ml ether, stirred 2 hr, left 24 hr at 0°, poured on ice and the ppt was collected by filtration, washed with ice-cold 2N HCl, 2N Na_2CO_3 and H_2O and dried *in vacuo*. The product (3·5 g) was crystallized from benzene–hexane, to yield IX, colorless blades, m.p. 119–121°; lit.[16] m.p. 121–122°; λ_{CO} 5·65, 5·84 μ. Acidification of the alkaline washings led to the recovery of 0·15 g VIII.

3′-*Benzyloxy*-5,7-*dihydroxy*-3,4′,5′-*trimethoxyflavone* (XI). A mixture of 0·54 g X[17] (2·74 mmole), 3·82 g IX (6·85 mmole) and 1·46 g of the K salt of VIII (4·40 mmole) (prepared by neutralization of an ethanolic soln of VIII with ethanolic KOH, followed by evaporation and drying at 80°) was heated at 175°/30 mm for 2 hr, then taken up in 30 ml 10% NaOHaq, refluxed 15 min, evaporated *in vacuo*, the residue was dissolved in 35 ml water and acidified with conc HCl. The ppt was digested on a steam-bath with 6% $NaHCO_3$aq and the residue (1 g) was crystallized from benzene to give XI as a yellow powder, m.p. 226–228°. (Found: C, 66·62; H, 5·06. $C_{25}H_{22}O_8$ requires: C, 66·66; H, 4·92%.)

3′-*Benzyloxy*-5-*hydroxy*-3,7,4′,5′-*tetramethoxyflavone* (XII). A soln of 0·41 g XI (0·91 mmole) in 20 ml dry acetone was treated with 2·2 g anhyd K_2CO_3 and 0·1 ml (1·10 mmole) of Me_2SO_4, refluxed 7 hr, filtered, evaporated *in vacuo*, and the residue was crystallized from MeOH to give 0·37 g XII, yellow prisms, m.p. 104–105°. (Found: C, 67·23; H, 5·32. $C_{26}H_{24}O_8$ requires: C, 67·23; H, 5·21%.)

[11] A. G. Perkin and J. J. Hummel, *J. Chem. Soc.* **69**, 1287 (1896).

[12] J. Kalff and R. Robinson, *J. Chem. Soc.* **127**, 181 (1925).

[13] F. Mauthner, *Liebigs Ann.* **449**, 102 (1926).

[14] F. Faltis and A. Troller, *Ber. Dtsch Chem. Ges.* **61**, 345 (1928).

[15] W. Meier and A. Fürst, *Helv. Chim. Acta* **45**, 232 (1962).

[16] J. L. Farkas, M. Nogradi and J. Strelinsky, *Tetrahedron Letters* 4563 (1965).

[17] Prepared according to W. K. Slater and H. Stephen, *J. Chem. Soc.* **117**, 309 (1920) by condensation of phloroglucinol with methoxyacetonitrile, which was obtained according to T. Malkin and R. Robinson, *J. Chem. Soc.* **127**, 369 (1925).

5,3′-Dihydroxy-3,7,4′,5′-tetramethoxyflavone (I). A soln of 0·11 g XII in 5·5 ml glacial AcOH and 3·5 ml conc HCl was heated 90 min on a steam-bath, then brought to pH 5–6 with 2N NaOH. The ppt was crystallized from MeOH: the micro-crystalline product (75 mg, m.p. 148–150°) was sublimed at 200°/1 mm to give I, m.p. 149–151°, whose IR spectrum was superimposable on that of the natural product and which did not depress its m.p.

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