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A XANTHONE FROM *GARCINIA MANGOSTANA*

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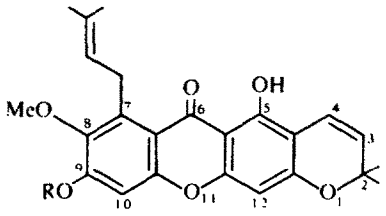
Key Word Index—*Garcina mangostana*; Guttiferae; xanthone; 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2H,6H-pyrano [3,2-*b*] xanthen-6-one.

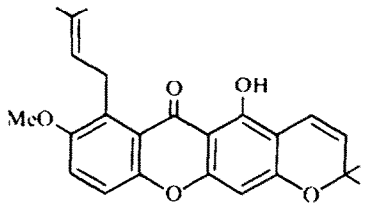
The fruit hulls of *Garcinia mangostana* L. are reported [1] to be used as an astringent and also used against cholera, dysentry and diarrhoea. The fruit hull of this plant has been reported to contain five polyoxygenated xanthones, mangostin **4** [2, 3], β-mangostin [4], nor-mangostin [5] gartanin and 8-desoxygartanin [6]. In this paper the isolation and characterization of a new polyoxygenated xanthone from the same plant are reported.

Petrol extraction of the dried and powdered fruit hulls followed by column chromatographic separation afforded another new xanthone in addition to several other known compounds. On the basis of some chemical and spectral data the structure of the new xanthone (**1**) was established as follows. Compound **1** mp 156–157° $C_{24}H_{24}O_6$ (M^+ at *m/e* 408) showed UV and IR spectra characteristic of a 1,3,6,7-tetraoxygenated xanthone [2–5]. The MS showed in addition to the molecular ion peak significant fragment ion peaks arising from the loss of Me and MeCO from the M^+ ion, suggesting [7] the presence of a methoxyl group *ortho* to the isopentenyl side chain. The ^{1}H NMR spectrum in $CDCl_3$ showed the 5-OH signal at δ 13.55 (1 H, *s*) and also showed signals at 6.64 (1 H, *d*), 5.47 (1 H, *d*) and at 1.40 (6 H, *s*) which indicated the presence of a 2,2-dimethyl chromene ring substitution at the xanthone ring, with the chromene double bond *ortho* to the C-5 hydroxyl. The ^{1}H NMR signals at 5.18 (1 H, *t*), 4.01 (2 H, *d*), 1.76 (3 H, *s*) and 1.62 (3 H, *s*) were the characteristic signals for an isopentenyl side chain *ortho* to the C-6 carbonyl. Finally the ^{1}H NMR signals at 6.73 (1 H, *s*) and at 6.15 (1 H, *s*) were attributed to H-10 and H-12 respectively, the latter proton of the electron-rich phloroglucinol ring being shifted upfield [8]. All these ^{1}H NMR shifts have been assigned on the basis of selective spin-decoupling experiments and by comparison with the reported [9–12] NMR data for xanthones.

The ^{13}C NMR chemical shifts for the natural xanthone (**1**) and its methylated derivative (**2**) are presented in Table 1, along with reported shifts for the closely related calabaxanthone [15]. The shifts were assigned on the basis of the noise-decoupled spectra of **1** and **2**, from the off-resonance and selective hetero-decoupling measurements on **2**, by comparison of the shifts of **1** and **2** with each other and with reported ^{13}C NMR data for flavonoids, coumarins [13, 14] and oxygenated xanthones [15–21] (especially those of calabaxanthone (**3**) [15]) and by application of known substituent shifts [15–21]. The shifts of all carbons, except for C-5, C-9, C-10a, C-11a and C-12a, are thus unambiguously assignable [however, the shifts for C-1′ and C-3′ (Me) in **1** remain reversible]. Preliminary selective hetero-decoupling measurements suggested that the chelated hydroxyl-carrying C-5 appeared at 157.8 (collapsing to a singlet in the presence of D_2O) and the methoxyl-bearing C-9 at 158.0 in **2**. Among other ^{13}C shifts which differ from those of calabaxanthone, the signals at 103.7 and 104.3 in **2** were assigned to C-5a and C-4a, respectively.



1 R = H
2 R = Me



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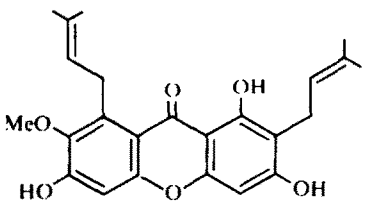

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Table 1. ^{13}C NMR chemical shifts of **1**, **2** and **3*** in ppm (±0.05) downfield from TMS in $CDCl_3$ solution and ^{13}C-^{1}H one bond couplings of **2** in Hz (±2)

	1	2	^1J	3*
C-2	77.8	77.8		77.9 78.4)‡
C-3	126.9	126.9 *d*†	164	115.8
C-4	115.6	115.6 *d*†	168	127.0–128.4)‡
C-4a	104.4	104.3		104.1
C-5	157.8§	157.8§		160.2
C-5a	103.6	103.7		104.1
C-6	181.8	181.8		183.2
C-6a	112.1	111.7		118.7
C-7	136.9	137.0		115.8
C-8	142.7	143.9		153.5
C-9	154.5§	158.0§		122.8
C-10	101.6	98.2 *d*	164	118.7
C-10a	155.6§	155.2§		151.7
C-11a	156.1§	156.0§		156.0
C-12	94.0	93.8 *d*	167	94.0
C-12a	159.8§	159.5§		158.0
C-2(Me)	28.3	28.2 *q*	127	28.4
C-8(OMe)	61.8	60.8 *q*	144	56.7
C-9(OMe)		55.9 *q*	146	
C-1′	26.5§	26.0 *tr*	130	25.8
C-2′	123.1	123.1 *dm*†	158	115.8
C-3′	131.8	131.5		131.9
C-4′	18.1	18.1 *q*	125	18.1
C-3′(Me)	25.6§	25.8 *q*	125	25.0

* Taken from ref. [15].
† Fine structure in off-resonance spectra.
‡ For other xanthones given in ref. [15].
§ Tentative assignment.

Ortho substituent effects due to OMe/OH cause an upfield shift of the C-8 signal to 143.9 in **2** and to 142.7 in **1**, and the C-10 signal to 98.2 in **2** and to 101.6 in **1** (cf. calabaxanthone **3**, 153.5 and 118.7 for C-8 and C-10, respectively). The C-6a signal in calabaxanthone at 118.7 was shifted upfield to 111.7 in **2** and to 112.1 in **1** due to the *para* substituent effects of OMe/OH. The 8-OMe signal was shifted downfield to 60.8 in **2** and to 61.8 in **1**, being flanked by two *ortho* substituents [18, 20–22]. Similarly, the prenyl side chain C-2′ signal was shifted downfield to 123.1 in **1** and **2** owing to increased steric congestion at that site.

Westerman *et al.* [15] assigned a signal at 115.8 to C-3 in **3**; no δ value was given for C-4 but other xanthones with a chromene ring showed 127.0–128.4 for carbon atoms corresponding to C-4. However, irradiation of the 3-H (at 5.53) changed the ^{13}C doublet at 126.9 to a singlet. Similarly, the 115.6 doublet became a singlet on irradiation of the 4-H (at 6.72). This suggests that the assignment of Westerman *et al.* for C-3 should be revised. A similar revision of 115.8 for C-7 in **3** [15] may be necessary because we now assign 136.9 in **1** and 137.0 in **2** to C-7. However, the application of shift increments to xanthones [16–21] may give unreliable results. It has been found that for *ortho* disubstituted anisoles and *ortho* methoxylated xanthones additivity fails which is explained by a perturbation of the interaction of the methoxy group with the aromatic ring [18, 22]. The *ortho* carbon atoms resonate in these cases usually some 9.5 ppm more downfield than predicted. If the assignment of Westerman *et al.* [15] is correct, then the C-7 shift of **1** and **2** as compared to **3** indicates that the introduction of a hydroxyl or methoxyl to C-9 inhibits the resonance of the 8-methoxy group with the aromatic ring so seriously that its shielding effect towards *ortho* C-7, usually 19 ppm upfield [18], totally disappears.

All the above NMR data, coupled with the shift of 26 nm in the UV maximum in the presence of fused NaOAc and a positive Gibbs test, strongly suggest structure **1** for the natural xanthone. Final confirmation was obtained by the cyclodehydrogenation [9] of mangostin (**4**) which afforded 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2H,6H-pyrano-[3,2-*b*]-xanthen-6-one as fine yellow crystals from C_6H_6, mp 158–159° (lit. [9] mp 166–168°) which was found to be identical (UV, IR and NMR) to the natural xanthone (**1**).

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REFERENCES

1. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) p. 123. *Glossary of Indian Medicinal Plants*. CSIR, India.
2. Schmid, W. (1855) *Ann.* **93**, 83.
3. Yates, P. and Stout, G. H. (1958) *J. Am. Chem. Soc.* **80**, 1691.
4. Yates, P. and Bhat, H. B. (1968) *Can. J. Chem.* **46**, 3770.
5. Jefferson, A., Quillinan, A. J., Scheinmann, F. and Sim, K. Y. (1970) *Aust. J. Chem.* **23**, 2539.

6. Govindachari, T. R., Kalyanaraman P. S., Muthkumaraswamy, N. and Pai, B. R. (1971) *Tetrahedron* **27**, 3919.
7. Stout, G. H., Krahn, M. M., Yates, P. and Bhat, H. B. (1968) *Chem. Commun.* 211.
8. Wolfrom, M. L., Komitsky, F. and Looker, J. H. (1965) *J. Org. Chem.* **30**, 144.
9. Locksley, H. D., Quilli an, A. J. and Scheinmann, F. (1971) *J. Chem. Soc. D:* Sec.-C 3804.
10. Marta, M., Delle Monache, F., Marini-Bettolo, G. B., Lyra, D. A., DeMello, J. F. and DeLima, O. G. (1976) *Gazz Chim. Ital.* **106**, 651.
11. Scheinmann, F. (1967) *Chem. Commun.* 1015.
12. Lins Mesquita, A. A., de Oliveira, W. G., Taveira Neiva, R. M. and Gottlieb, O. R. (1975) *Phytochemistry* **14**, 803.
13. Wehrli, F. W. (1975) *J. Chem. Soc. Chem. Commun.* 663.
14. Chang, C. J., Floss, H. G. and Steck, W. (1977) *J. Org. Chem.* **42**, 1337.
15. Westerman, P. W., Gunasekera, S. P., Uvais, M., Sultanbawa, S. and Kazlauskas, R. (1977) *Org. Magn. Reson.* **9**, 631.
16. Idris, M. Sum b. Hj., Jefferson, A. and Scheinmann, F. (1977) *J. Chem. Soc. Perkin Trans 1:* 2158.
17. Frahm, A. W. and Chaudhuri, R. K. (1979) *Tetrahedron* **35**, 2035.
18. Chaudhuri, R. K., Zymalkowski, F. and Frahm, A. W. (1978) *Tetrahedron* **34**, 1837.
19. Sundholm, E. G. (1979) *Acta Chem. Scand. Ser. B* **33**, 475.
20. Sundholm, E. G. (1978) *Acta Chem. Scand. Ser. B* **32**, 177.
21. Huneck, S. and Hofle, G. (1978) *Tetrahedron* **34**, 2491.
22. Dhami, K. S. and Stothers, J. B. (1966) *Can. J. Chem.* **44**, 2855.

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THE GENETIC CONTROLLED HYDROXYLATION PATTERN OF THE ANTHOCYANIN B-RING IN *SILENE DIOICA* IS NOT DETERMINED AT THE *p*-COUMARIC ACID STAGE

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Key Word Index—*Silene dioica*; Caryophyllaceae; red campion; anthocyanin biosynthesis; hydroxylation; genetic control; *p*-coumaric acid; caffeic acid.

INTRODUCTION

The basic C-15 skeleton of flavonoids is formed by the head-to-tail condensation of three malonyl-CoA units to one molecule of hydroxycinnamoyl-CoA [1–4]. The A-ring and part of the heterocyclic ring are derived from the acetate units, the B-ring from the hydroxycinnamoyl ester. The basic C-15 flavonoid is then further modified to yield the various flavonoid classes [5]. In this biosynthetic pathway, the hydroxylation pattern of the B-ring can either be determined at the C-9 level, by starting with the appropriate hydroxycinnamoyl-CoA ester [6], or at the C-15 level, by hydroxylation of one of the C-15 intermediates [7].

In *Silene dioica* the hydroxylation pattern of the B-ring of the anthocyanidin molecule, and of the acyl group bound to the terminal sugar at the 3-position, is controlled by gene *P*, whereas the binding of the acyl group is governed by gene *Ac*. The hydroxylation pattern of the acyl group, however, corresponds with that of the B-ring of the anthocyanidin molecule. Thus in *p/p Ac/Ac* plants, in which only pelargonidin glycosides are found, the acyl group is *p*-coumaric acid. In *P/P Ac/Ac* plants the anthocyanidin is cyanidin, and the acyl group is caffeic acid [8, 9]. This suggests that homozygous recessive *p/p* plants are unable to synthesize caffeic acid, which is used both as a precursor in the biosynthesis of the anthocyanidin molecule and for acylation.

In this paper, we have investigated whether gene *P* is involved in the conversion of *p*-coumaric to caffeic acid.

RESULTS AND DISCUSSION

The enzyme catalysing the hydroxylation of *p*-coumaric acid to caffeic acid was present in petals and leaves of *Silene dioica*. Most of the activity is lost by polyclar AT (PVP) chromatography or by gel filtration. Therefore the supernatant of the crude homogenate was used to study the properties of the enzyme. The amount of caffeic acid formed was proportional to time, for periods