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# XANTHONES IN THE HEARTWOOD OF *CALOPHYLLUM INOPHYLLUM*: A GEOGRAPHICAL SURVEY*

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**Abstract**—The heartwood constituents of an Australian specimen of *C. inophyllum* L., are shown to be jacareubin, 1,7-dihydroxyxanthone (euxanthone), 1,5,6-trihydroxyxanthone, 1,6-dihydroxy-5-methoxyxanthone (buchanaxanthone), 6-desoxyjacareubin, 2-(3,3-dimethylallyl)-1,3,5-trihydroxyxanthone and 2-(3,3-dimethylallyl)-1,3,5,6-tetrahydroxyxanthone (the latter two isolated as their methyl ether derivatives). These metabolites are compared with those obtained from specimens of the same species collected in Malagasy and in India.

RECENTLY we reported[1] the presence of 6-desoxyjacareubin (I), jacareubin (II), and 2-(3,3-dimethylallyl)-1,3,5,6-tetrahydroxyxanthone (III) [the putative biogenetic precursor of jacareubin (II)] in a sample of *C. inophyllum* L., collected in the Malagasy Republic (formerly Madagascar). This study was of interest since earlier Govindachari *et al.*,[2] working with a heartwood sample of the same species collected near Madras, India, had isolated two different xanthones, namely, 1,5,6-trihydroxyxanthone (IV) (mesuaxanthone B), and 6-(3,3-dimethylallyl)-1,5-dihydroxyxanthone (V) (calophyllin B), in addition to two unidentified metabolites, neither of which was jacareubin (II).[3] From a chemotaxonomic viewpoint, the absence of jacareubin (II) from the Indian specimen is curious because until now the heartwood of every *Calophyllum* species has been shown to contain substantial quantities of this metabolite. Indeed its ubiquity makes jacareubin (II) a reliable chemotaxonomic marker for the genus.†[4]

The disparity between the metabolites reported for the Indian and the Malagasy specimens of *C. inophyllum* L., may be explained in two ways, either, (a) the identification of the species is erroneous in either or both cases, or (b) geography exerts a more profound influence on the nature of the metabolites than would otherwise be expected within a species.

As both research groups disclaimed the likelihood of an error in their identification of *C. inophyllum* L.,[3] explanation (a) was considered doubtful. The arrival of a sample of the heartwood of *C. inophyllum* L., from Australia enabled us to test more fully the alternative explanation (b) based on geographical origin.

* Part XVII in the series "Extractives from Guttiferae"; for Part XVI see H. D. LOCKSLEY and I. G. MURRAY, *J. Chem. Soc.* (C) 392 (1970).

† Additionally, with the sole exception of *C. brasiliense* Camb,, every *Calophyllum* species so far investigated also contains the putative precursor (III) of jacareubin (II).

[1] B. JACKSON, H. D. LOCKSLEY and F. SCHEINMANN, *Phytochem.* **8**, 927 (1969).

[2] T. R. GOVINDACHARI, B. R. PAI, N. MUTHUKUMARASWAMY, U. R. RAO and N. NITYANANDA RAO, *Indian J. Chem.* **6**, 57 (1968).

[3] T. R. GOVINDACHARI, personal communications.

[4] I. CARPENTER, H. D. LOCKSLEY and F. SCHEINMANN, *Phytochem.* **8**, 2013 (1969).

The heartwood of the Australian specimen, extracted and worked up in a manner similar to that previously reported,[1] was again found to contain substantial quantities of both jacareubin (II) and the precursor (III) [isolated as its trimethyl ether (VI)], in addition to other xanthones commonly encountered in species belonging to the genus *Calophyllum*,[4] namely, 1,7-dihydroxyxanthone (VII) (euxanthone), 1,5,6-trihydroxyxanthone (IV), 1,6-dihydroxy-5-methoxyxanthone (VIII) (buchanaxanthone), 6-desoxyjacareubin (I), and 2-(3,3-dimethylallyl)-1,3,5-trihydroxyxanthone (IX), the putative biogenetic precursor of 6-desoxyjacareubin (I), which was isolated as its dimethyl ether derivative (X).

Although the Australian specimen of *C. inophyllum* L., appears to contain a wider array of xanthones than the Malagasy one, the presence in both of substantial quantities of jacareubin (II) and the precursor (III) is particularly significant since it indicates that these two chemotaxonomic markers can survive in the species despite a geographical separation of some 5000 miles.

It now becomes of interest to chemotaxonomy to know whether the absence of jacareubin (II) and/or its precursor (III) from the Indian specimen is an isolated observation: an examination of other specimens of the species indigenous to the Indian subcontinent might help to clarify this point.

## EXPERIMENTAL

I.r. spectra were recorded as Nujol mulls unless otherwise stated. Analytical and preparative TLC were carried out on silica gel G, Stahl (Merck). M.ps are uncorrected. TLC comparisons against authentic compounds were carried out initially under u.v. light and then visualized by spraying with aq. $FeCl_3$

*Extraction of* Calophyllum inophyllum *L.*

A sample of the heartwood of *C. inophyllum* L., (identification number 8281) was collected in Queensland by officers of the C.S.I.R.O., Melbourne, Australia. Shavings of the heartwood (1·5 kg) were Soxhlet extracted first with $CHCl_3$ for 5 days, then with acetone for 3 days. The resulting extracts, on evaporation, gave straw coloured amorphous solids (11·6 and 7·2 g, respectively) which, on TLC examination, were found to contain the same metabolites and were consequently combined.

(i) *Chromatographic separation of the combined extracts.* The combined extracts (18·4 g) were triturated with $CHCl_3$ and the filtrate introduced onto a column of silica gel (1·35 kg) prepared in $CHCl_3$. The undissolved residue (3 g) was found to contain (TLC) the same constituents as those in the filtrate and was not further examined. Elution with $CHCl_3$ followed by increasingly polar mixtures of $CHCl_3$–EtOAc gave several fractions some of which contained (TLC) more than one component. Fractions were combined as appropriate to their constituents to produce three composite fractions (1–3) which are discussed below in the order of their elution from the column.

(ii) *Isolation of jacareubin (II).* Fraction 1 (eluted with EtOAc–$CHCl_3$, 1:19, 2:18 and 3:17) gave a pale yellow solid (6·2 g) imparting a green colour to $FeCl_3$. TLC examination showed it to consist mainly of jacareubin, and recrystallization from EtOAc–light petroleum (b.p. 60–80°) furnished jacareubin (II) as yellow plates (2·71 g), m.p. 255–256° (lit.,[1,5] m.p. 254–256°), identical with an authentic specimen [mixed m p., i.r. spectra, and TLC comparison ($R_f$ 0·45 in HOAc–$CHCl_3$, 1:9)].

The dimethyl ether (XI), prepared by methylation of jacareubin (100 mg) with ethereal $CH_2N_2$, was crystallized from acetone–light petroleum (b p. 60–80°) to give pale prisms (63 mg), m p. 189–191°, identical with an authentic sample of jacareubin dimethyl ether (XI) (lit.,[6–8] m.ps 192° and 194°) [mixed m p., i r. spectra and TLC comparison ($R_f$ 0·75 in $CHCl_3$–benzene, 7·3 and $R_f$ 0·77 in EtOAc–benzene, 1:9)].

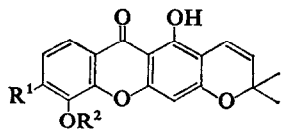
(iii) *Isolation of euxanthone (VII).* Fraction 2 (eluted with EtOAc–$CHCl_3$, 1·19) was combined with the mother liquors from the crystallization of jacareubin [see (ii)]. Removal of the solvent and crystallization of the residue from EtOAc gave a solid (3·5 g). Subsequent recrystallizations from this solvent and from

[5] A. JEFFERSON and F. SCHEINMANN, *J. Chem. Soc.* (C) 175 (1966)
[6] F. E. KING, T. J. KING and L. C. MANNING, *J. Chem. Soc.*, (a) 3932 (1953); (b) 563 (1957).
[7] B. JACKSON, H. D. LOCKSLEY and F. SCHEINMANN, *J. Chem Soc* (C) 178 (1966)
[8] B. JACKSON, H. D LOCKSLEY and F. SCHEINMANN, *J. Chem. Soc* (C) 2500 (1967).

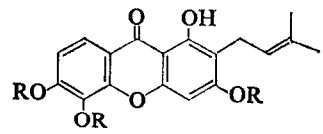
toluene gave euxanthone (VII) as yellow prisms (250 mg) m.p. 238° (lit.,[9–13] m.p. 239°), identical with an authentic sample [mixed m.p., i.r. spectra, and TLC comparison ($R_f$ 0·5 in EtOAc–$CHCl_3$, 1:9)].

The mother liquors from the purification of euxanthone were combined (2·94 g) and part of the mixture (1·24 g) was resolved into its four components by preparative TLC using HOAc–$CHCl_3$ (1:9) as eluent. One band (orange-brown under u.v.) had the same characteristics as those of euxanthone (VII) and on work up it yielded a further quantity of this metabolite (124 mg). The following three major metabolites were also isolated.

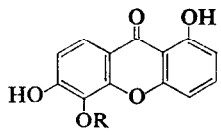
(a) *Isolation of* 1,5,6-*trihydroxyxanthone* (*IV*). The material from the band at $R_f$ 0·75 (brown under u.v.) was isolated quickly to avoid aerial oxidation and the metabolite was then crystallized from EtOAc–light petrol (b.p. 60–80°) to give the title compound (338 mg) as a yellow powder, m.p. 280–283° (decomp.) (lit.,[8,10–14] m.p. 285–286°), identical with an authentic sample [mixed m.p., i.r. spectra (as KBr discs) and TLC comparison $R_f$ 0·6 in HOAc–$CHCl_3$ (15:85) and $R_f$ 0·4 in EtOAc–$CHCl_3$ (20:80)].



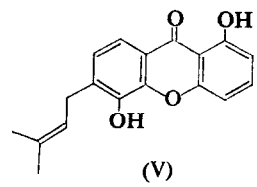
(I) $R^1 = H, R^2 = H$
(II) $R^1 = OH$ $R^2 = H$
(XI) $R^1 = OMe$ $R^2 = Me$



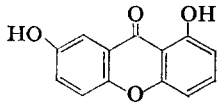
(III) R=H
(VI) R=Me



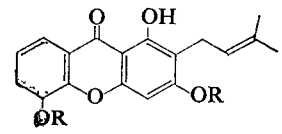
(IV) R=H
(VIII) R=Me



(V)



(VII)



(IX) R=H
(X) R=Me

(b) *Isolation of buchanaxanthone* (*VIII*). The material from the band at $R_f$ 0·85 (orange under u.v.) was isolated as a yellow solid (414 mg) and crystallized several times from benzene (with charcoal) to furnish buchanaxanthone (VIII) as cream needles (180 mg) m.p. 244° (lit.,[12,14] 243–246° and 243–245°), identical with an authentic sample [mixed m.p., i.r. spectra, and TLC comparison $R_f$ 0·6 in EtOAc–$CHCl_3$ (1:9)].

[9] D. B. Spoelstra and M. J. van Royen, *Rec. Trav. Chim.* **48**, 370 (1929).
[10] H. D. Locksley, I. Moore and F. Scheinmann, *J. Chem. Soc.* (c) 430 (1966).
[11] B. Jackson, H. D. Locksley and F. Scheinmann, *J. Chem. Soc.* (c) 2201 (1969).
[12] H. D. Locksley and I. G. Murray, *J. Chem. Soc.* (c), 1567 (1969).
[13] I. Carpenter, H. D. Locksley and F. Scheinmann, *J. Chem. Soc.* (c) 2421 (1969).
[14] B. Jackson, H. D. Locksley, I. Moore and F. Scheinmann, *J. Chem. Soc.* (c) 2579 (1968).

(c) *Isolation of 6-desoxyjacareubin (I).* The material from the band at $R_f$ 0·9 (black under u.v.) was isolated and yielded a solid (75 mg) which was further purified by preparative TLC to give 6-desoxyjacareubin (I) (45 mg) as yellow cubes, m.p. 213° (lit.,[8,12] m.ps 211–213° and 212–214°) from EtOAc, identical with an authentic sample [mixed m.p., i.r. spectra and TLC comparison ($R_f$ 0·8 in EtOAc–$CHCl_3$, 1:9)].

(d) *Isolation of 2-(3,3-dimethylallyl-1,3,5,6-tetrahydroxyanthone (III) as its trimethyl ether (VI).* Fraction 3 from the column chromatogram (1·20 g) (eluted with EtOAc–$CHCl_3$, 1:4, 1:3, 3.7 and 5:7) was largely a mixture of two components with $R_f$ values of 0·5 and 0·8 (TLC in HOAc–$CHCl_3$, 1:3).

Methylation of the crude mixture with an excess of ethereal $CH_2N_2$ was necessary to minimize aerial oxidation during isolation. Recrystallizations of the methylated product using ethanol gave pure 2-(3,3-dimethylallyl)-1-hydroxy-3,5,6-trimethoxyxanthone (VI) (500 mg) as yellow needles, m.p. 170·5° (lit.,[7,8,12,15] m.p. 164–165° and 166–167°) identical with an authentic sample [mixed m.p., i.r. spectra, and TLC comparison ($R_f$ 0·7 in ethyl acetate–benzene, 1:9)]. The second main component of the methylated fraction possessed the same TLC characteristics as those of an authentic sample of jacareubin dimethyl ether (XI) [see (ii) above].

TLC comparisons using authentic compounds revealed that the two main components in fraction 3, prior to its methylation, are 2-(3,3-dimethylallyl)-1,3,5,6-tetrahydroxyxanthone (III) and jacareubin (II).

The mother liquors remaining from the purification of the trimethyl ether (VI) were examined by TLC and in addition to jacareubin dimethyl ether (XI) were found to contain a small quantity of a third component Several recrystallizations from EtOAc–light petroleum (b.p. 60–80°) gave 2-(3,3-dimethylallyl)-1-hydroxy-3,5-dimethoxyxanthone (X) (14 mg), m p. 166–168° (lit.,[8,16] m.ps 167–170° and 172–173°) identical with an authentic sample [mixed m.p., i.r. spectra and TLC comparison ($R_f$ 0·8 in EtOAc–benzene, 1 9)]. TLC investigation showed that the dimethyl ether (X) was not present in the original unmethylated fraction 3: the original metabolite is probably the parent phenol (IX).

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[15] I. CARPENTER, H. D. LOCKSLEY and F. SCHEINMANN, *J. Chem. Soc.* (C) 486 (1969).
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