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A NEW ACRIDONE ALKALOID FROM *CITRUS DECUMANA*

SUDAM C. BASA* and RABINDRA N. TRIPATHY

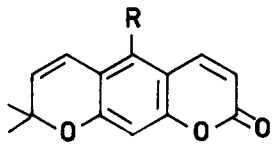
Regional Research Laboratory, Bhubaneswar-751 013, India

ABSTRACT.—From the root bark extracts of *Citrus decumana* two coumarins, xanthyletin (**1**) and xanthoxyletin (**2**), a new acridone alkaloid, 2′,2′-dimethyl-(pyrano 5′, 6′:3:4)-1,5-dihydroxy, 6-methoxy, 10-methyl acridone (**3**), and another known alkaloid, 2′,2′-dimethyl-(pyrano 5′,6′:3:4)-1-hydroxy-5,6-dimethoxy, 10-methyl acridone (**6**), have been isolated and their structures established by spectral and chemical methods. Cmr data of the alkaloids are reported.

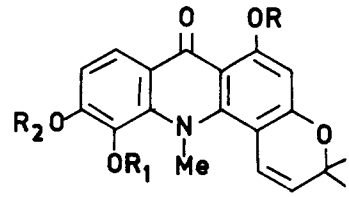
Acridones are characteristic secondary metabolites of the Rutaceae, and their occurrence in higher plants is restricted to this botanical family. The number of compounds of this class has increased considerably in recent years (1,2).

We report here the isolation and structure determination of a new acridone alkaloid as well as another one reported recently (3), two closely related coumarins, and β-sitosterol from *Citrus decumana* L. (Rutaceae) collected near Calcutta, India, in October 1979.

Although the flavonoids and coumarins in *C. decumana* were described previously (4-7), this is the first report of acridones from this species.



1 R=H
2 R=OMe



3 $R=R_1=H$, $R_2=Me$
4 $R=H$, $R_1=COCH_3$, $R_2=Me$
5 $R=R_1=COCH_3$, $R_2=Me$
6 $R=H$, $R_1=R_2=Me$
7 $R=R_1=R_2=Me$
8 $R=R_2=H$, $R_1=Me$

RESULTS AND DISCUSSION

A concentrated hexane extract of *C. decumana* roots on column chromatography furnished two crystalline solids in the early fractions. The identification of the two solids as xanthyletin (**1**) and xanthoxyletin (**2**) was based on the spectroscopic data and their comparison with authentic samples (see Experimental section). Incidentally, *C. decumana* is another species in which these two closely related coumarins occur, the other species being *Zanthoxylum americanum* (8), *Chloroxylon swietinia* (9,10), and *Afraegle paniculata* (11), all belonging to the Rutaceae. Xanthyletin also occurs in the closely related species *Citrus depressa* (3).

On further elution of the column with C_6H_6-$CHCl_3$ (80:20) as solvent, a deep yellow solid was isolated, and this solid was subjected to further Si gel preparative tlc when the purified compound was obtained.

This compound (**3**) had mp 260° and uv spectrum bands at λ max 205, 268, 277, 340, and 390 nm (log ε; 4.26, 4.45, 4.42, 3.87, and 3.84) characteristic of a 9-acridone system (12,13). It furnished a monoacetate with pyridine and Ac_2O at room temperature, and methylation with CH_2N_2 furnished a monomethyl ether. Both of these

derivatives showed a green ferric reaction, indicating the presence of a *peri*-hydroxyl group, as has been observed with noracronycine (14).

The ir spectrum of the compound showed bands at 1625, 1580, and 1550 cm^{-1}, strongly reminiscent of an acridone nucleus. The complete structure of the new alkaloid was evident from its pmr and ms data. The latter exhibits its most intense peak at *m/z* 338 (M-15), which is characteristic of benzopyrilium ions from 2,2-dimethyl chromenes (15,16). The pmr spectrum (Table 1) showed the following signals: a sharp singlet at 1.48 δ (6H, S) together with the doublets at 5.56 and 6.48 δ (each 1H, J=9 c/s) are typical of a 2,2-dimethylchromene system (15,17). Two sharp singlets at 3.68 and 3.84 δ (each 3H, S) were due to an -NMe and a -OMe group. A sharp singlet at 6.18 δ (1H) was due to the aromatic proton at C-2, consistent with the observation of Pakrashi *et al.* (18) that such rather far-upfield shifts have been noted with single aromatic protons flanked by oxygen atoms or by nitrogen and oxygen. Two doublets at 6.96 and 7.96 δ (each 1H, J=9 c/s) were assigned to the C-7 and C-8 protons, respectively, and this downfield appearance of the C-8 proton excludes the possibility of the substituents being placed at C-7 and C-8. The OH proton at C-5 was observed as a broad signal around 9.6 δ, and the far-downfield signal at 14.40 δ (1H, s) was due to the strongly H-bonded phenolic proton. The ms of the alkaloid showed M^+ at *m/z* 353 compatible with the molecular formula $C_{20}H_{19}NO_5$. The base peak at *m/z* 338 (M-15) and the fragmentation pattern was typical of acridone alkaloids: loss of $-CH_3$ from the 2,2-dimethylchromene system followed by loss of CO from ring B.

The diacetyl derivative of the alkaloid **5**, prepared by heating it with pyridine and Ac_2O, proved to be of much help. The diacetate $C_{24}H_{23}NO_7$ (M^+ 437) had a mp of 217°. In addition to the pmr signals of the parent alkaloid, barring the OH signals, the diacetate derivative showed two new signals for two acetoxy methyl functions at 2.40 and 2.48 δ and the C-2 proton moved downfield and appeared at 6.46 δ, thus proving the presence of an OH group at C-1. Moreover, the doublets for the C-7 and C-8 protons remained unaffected, thereby indicating that the OMe group needed to be placed at C-6 and, consequently, the OH group at C-5. Recently, citracridone I (**8**), mp 275-278°, bearing an OH group at C-6 and an OMe group at C-5, has been isolated from *C. depressa* (3). Acetylation of the alkaloid with pyridine and Ac_2O at room temperature furnished a mono acetate (**4**), $C_{22}H_{21}NO_6$ (M^+ 395), which had mp 210°. Its pmr spectrum showed one acetoxy methyl at 2.43 δ, and the downfield proton was visible at 14.3 δ.

The alkaloid on treatment with ethereal CH_2N_2 at room temperature gave a monomethyl ether (**6**), $C_{21}H_{21}NO_5$ (M^+ 367), mp 159°. Its pmr indicated the appearance of a new signal at 4.02 δ for the methoxyl function. To add to this, the resonance positions of the OMe carbons in compound **6** (Table 2) clearly support the placement of the OH group at C-5 and that of the OMe at C-6. This derivative (**6**) has been isolated as a natural product from *C. depressa* (3). Reaction of the alkaloid with methyl iodide at room temperature furnished a dimethyl ether (**7**) $C_{22}H_{23}NO_5$ (M^+ 381), mp 180°. Pmr of this derivative showed two additional signals at 3.98 and 4.02 δ, thus confirming the presence of two phenolic hydroxyls in the parent base.

Preparative tlc of the C_6H_6-$CHCl_3$ (90:10) eluates over Si-gel (C_6H_6-EtOAc, 19:1) furnished a new alkaloid $C_{21}H_{21}NO_5$ (M^+ 367), mp 159°, which showed uv spectrum bands at λ max 268, 280, and 339 (log ε, 4.44, 4.40, and 3.89) and also a green ferric reaction. Its pmr indicated that it might be the monomethyl ether **6** of the alkaloid isolated from the C_6H_6-$CHCl_3$ (80:20) eluates. Its identity was established from its mmp, superimposable ir, and co-tlc. Cmr resonances of the alkaloids have been assigned and are recorded in the Table 2. A point of interest of this study is that the *N*-methyl carbons in both the alkaloids resonate at a rather low field (~48 δ). This is possibly due to

TABLE 1. ^{1}H-Chemical Shifts (δ: 100 MHz) of Alkaloids **3**, **6**, and Derivatives of **3**[a]

Compound	1-OR (R=H, CH_3, $COCH_3$)	2-H (1H)	5-OR (R=H, CH_3, $COCH_3$)	6-OMe (3H)	7-H (1H)	8-H (1H)	10-CH_3 (3H)	Others
3	14.4	6.18	9.6 br	3.84	6.96(d) (*J*9)	7.96(d) (*J*9)	3.68	1.48 (6H,8′,9′ Me) 5.56 (d,*J*9,6′H) 6.48 (d,*J*9,5′H)
4	14.3	6.18	2.43	3.86	7.05(d) (*J*9)	8.08(d) (*J*9)	3.62	1.52 (6H,8′,9′ Me) 5.66 (d,*J*9,6′H) 6.60 (d,*J*9,5′H)
5	2.48	6.46	2.40	3.88	7.0(d) (*J*9)	8.00(d) (*J*9)	3.66	1.52 (6H,8′,9′ Me) 5.66 (d,*J*9,6′H) 6.60 (d,*J*9,5′H)
6	14.3	6.22	4.02	3.90	6.98(d) (*J*9)	8.04(d) (*J*9)	3.75	1.52 (6H,8′,9′ Me) 5.54 (d,*J*9,6′H) 6.60 (d,*J*9,5′H)
7	4.02	6.20	3.98	3.92	6.96(d) (*J*9)	7.98(d) (*J*9)	3.72	1.52 (6H,8′,9′ Me) 5.52 (d,*J*9,6′H) 6.60 (d,*J*9,5′H)
8	14.52	6.23	3.91	9.33	7.00(d) (*J*9)	8.01(d) (*J*9)	3.75	1.53 (6H,8′,9′ Me) 5.61 (d,*J*10,6′H) 6.63 (d,*J*10,5′H)

[a]TMS as internal standard; unless otherwise stated all signals are singlets; coupling constants (*J*) are given in Hz.

TABLE 2. Cmr Assignments[a] of Alkaloid **3** and **6**

	3[b]	**6**[c]
C(1)	160.3	161.1
C(2)	97.3	98.2
C(3)	163.9	164.5
C(4)	102.2	102.4
C(4a)	147.1	147.7
C(4b)	142.5	142.3
C(5)	136.9	159.8
C(6)	156.5	157.4
C(7)	113.7	108.2
C(8)	121.8	122.4
C(8a)	120.4	120.7
C(9)	180.7	181.7
C(9a)	105.9	106.7
C(5′)	116.7	118.8
C(6′)	124.3	124.1
C(7′)	76.5	76.5
C(8′)	26.8	27.2
C(9′)	26.8	27.2
N-CH_3	48.6	48.9
O-CH_3	56.8	—
		56.8, C(6)
O-CH_3	—	59.6, C(5)

[a]Chemical shifts in δ relative to TMS
[b]In $CDCl_3$-($^2H_6$) DMSO (9:1)
[c]$CDCl_3$

the steric compression with C_5-OH and C_4-dimethylallyl group. A similar observation has also been reported recently (19). The upfield shift of the C_5 carbon in **6** compares well with the corresponding data in the xanthone series (20).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Each mp was determined on a Büchi apparatus and is uncorrected. Uv and ir spectra were measured on Varian 634 and Perkin-Elmer 337 instruments respectively. Pmr spectra were obtained on a JEOL PS-100 spectrometer with TMS as internal standard. Cmr spectra were taken on a JEOL PS-100 instrument equipped with a pulsed FT system operating at 25.15 MHz or on a JEOL FX-60 spectrometer operating at 15.00 MHz with deuterium internal lock and TMS as internal reference. Column chromatography was performed on BDH (British Drug House) silica gel.

PLANT MATERIAL.—The roots of *C. decumana* were collected 50 km from Calcutta and were identified at the Indian Botanic Garden, Howrah. A voucher specimen is kept in the herbarium.

EXTRACTION AND PURIFICATION.—The air-dried plant material (3.2 kg) was extracted with cold hexane (10 liters) for 7 days. The extract was concentrated under vacuum to give a thick, oily residue (13.86 g), and the resulting concentrated extract was separated first by column chromatography (Si gel) and further by repeated preparative tlc (Si gel). Known compounds were identified by comparing their pmr and ms with those of authentic samples.

ISOLATION OF COUMARINS.—Hexane-C_6H_6 (1:1) eluates on evaporation furnished a solid, mp 124-125°. This was subjected to Si gel preparative tlc using C_6H_6-EtOAc (19:1) as solvent. The two solids that were separated were characterized as xanthyletin (**1**) and xanthoxyletin (**2**).

Xanthyletin (**1**).—Compound **1** was crystallized from Me_2CO as thick needles; mp 128°. Its identity was established from its mmp and superimposable ir with an authentic sample.

Xanthoxyletin (**2**).—This compound was crystallized from MeOH as fine needles; mp 131°; pmr δ 1.45 (6H, S, 2 x CH_3), 5.63 (1H, d, J=10 Hz, 3′H), 6.16 (1H, d, J=9.5 Hz, 3-H), 6.50 (1H, S, 8-H), 6.55 (1H, d, J=10 Hz, 4′-H), 7.88 (1H, d, J=9.5 Hz, 4H); ms *m/z* 258 (M^+), 243, 228, 213, and 185.

ISOLATION OF β-SITOSTEROL.—The early C_6H_6 eluates on evaporation left a greenish-white solid

which, on repeated crystallization from MeOH, gave a white crystalline material, mp 137°. This was identified as β-sitosterol mmp and superimposable ir spectra with an authentic sample.

ISOLATION OF ALKALOID (**3**).—The C_6H_6-$CHCl_3$ (4:1) eluates on evaporation furnished a deep yellow solid (220 mg). The solid was subjected to repeated preparative tlc with C_6H_6-EtOAc (19:1) as solvent, where a yellow solid crystallized from MeOH, mp 260° (Found: C, 68.12, H, 5, 49; N, 3.97. $C_{20}H_{19}NO_5$ requires C, 67.98, H, 5.38; N, 3.96%). It had λ max (EtOH) 205, 268, 277, 340, and 390 nm (log ε, 4.26, 4.45, 4.42, 3.87, and 3.84); ν max (Nujol) 3400, 1625, 1580 cm^{-1}; *m/z* 353 (M^+), 338 (100%), 323, 322, 308, 294, 280, 177, and 162.

Acetylation of alkaloid **3**.—(A) Monoacetate of Alkaloid **3**: Acetylation of alkaloid **3** (28 mg) in pyridine (0.5 ml) and Ac_2O (1.5 ml) overnight at room temperature with usual work up, afforded the monoacetyl derivative **4**, lemon-yellow crystals (MeOH), mp 210° (Found: C, 66.90; H, 5.45; $C_{22}H_{21}NO_6$ requires C, 66.83; H, 5.31%); ν max (Nujol) 3400, 1760, and 1640 cm^{-1}; *m/z* 395 (M^+), 380 (100%), 338, and 322.

(B) Diacetate of Alkaloid **3**: Refluxing **3** (25 mg) in pyridine (0.5 ml) and Ac_2O (1.5 ml) at 110° for 4h with standard work-up and preparative tlc gave yellow diacetyl derivative (**5**), mp 217° (C_6H_6) (Found: C, 65.81; H, 5.33 $C_{24}H_{23}NO_7$ requires C, 65.90; H, 5.26%); ν max (Nujol) 1765, 1630 cm^{-1}, *m/z* 437 (M^+), 395, 380 (100%), 338, and 322.

Methylation of alkaloid **3** *with* CH_2N_2.—To a solution of the alkaloid **3** (30 mg) in MeOH (2 ml), ethereal CH_2N_2 (250 mg) was added. The resulting solution was kept at room temperature for 24 h and then the solvent removed. The resulting crude solid was recrystallized from EtOH to give yellow needles, mp 159° (Found: C, 68.59; H, 5.85; $C_{21}H_{21}NO_5$ requires C, 68.66; H, 5.72%); ν max (Nujol) 3400, 1635 cm^{-1}; *m/z* 367 (M^+).

Methylation of alkaloid **3** *with methyl iodide*.—To a solution of the alkaloid **3** (20 mg) in dry Me_2CO (5 ml) methyl iodide (1 ml) and anhydrous K_2CO_3 (300 mg) were added and stirred at room temperature for 96 h, the course of the reaction being followed by tlc. Usual work-up followed by preparative tlc gave a solid mp 180° (C_6H_6). (Found: C, 69.38; H, 6.15, $C_{22}H_{23}NO_5$ requires C, 69.29 and H, 6.03%) ν max (Nujol) 1640 cm^{-1}; *m/z* 381 (M^+), 366 (100%), 352, and 336.

ISOLATION OF ALKALOID (**6**).—The C_6H_6-$CHCl_3$ (9:1) eluates from the Si gel column, on evaporation, left a sticky mass which showed three spots on tlc. This was subjected to preparative tlc over Si gel (C_6H_6-EtOAc, 19:1) when the most intense band having Rf 0.65 gave a solid mp 159° (EtOH). Its pmr, co-tlc and mmp revealed its identity with the monomethylether of alkaloid **3**.

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