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Isolation and Structures of Citropone-A, -B, and -C from *Citrus* Plants, the First Examples of Naturally Occurring Homoacridone Alkaloids Containing a Seven-Membered Ring System

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The first isolation of the unique homoacridone alkaloids, citropone-A (1), -B (2), and -C (3) from the root or stem bark of some cultivated *Citrus* plants is described. The structure of citropone-A (1) was established unequivocally by spectrometric and single-crystal X-ray analyses, while citropone-B, which has the same skeleton as 1, was assigned structure 2 by comparison of the ^{1}H- and ^{13}C-nuclear magnetic resonance spectra with those of 1. The structure of citropone-C was proposed as formula 3 on the basis of spectrometric analyses using H–C long-range two-dimensional correlation spectroscopy and heteronuclear multiple bond connectivity spectrometries.

Keywords *Citrus*; Rutaceae; citropone; acridone; tropone; seven-membered ring; homoacridone; X-ray

Since the first isolation of acridone alkaloids from plants of the genus *Citrus* (Rutaceae) by us,[1] the occurrence of many kinds of acridone alkaloids from *Citrus* plants has been reported.[2,3] As a continuation of our studies on chemical constituents from this source,[1,2,4] we here describe the first isolation and structural elucidation of three homoacridone alkaloids named citropone-A (**1**), -B (**2**), and -C (**3**), having an unique seven-membered ring system in their molecules.[5]

Citropone-A (**1**) and -B (**2**) were obtained from root bark of *C. grandis* OSBECK f. *buntan* HAYATA collected in Taiwan and found to have a substituted tropone ring system in their molecules. Citropone-A (**1**) was also obtained from roots of *C. natsudaidai* HAYATA cultivated in Japan. Citropone-C (**3**) was isolated from roots of several hybrid seedlings resulting from crosses of [*C. unshiu* MARC. (miyagawa-wase) × *C. sinensis* OSBEC. (trobita orange)] (kiyomi) × *C. iyo* HORT. *ex* TANAKA (iyo), *C. flaviculpus* HORT. *ex* TANAKA (ogonkan) × *C. Tamurana* HORT. *ex* TAKAHASHI (hyuganatsu), and *C. paradisi* MACF. (dunkan-grapefruit) × *C. sinensis* OSBECK (hamlin), and stem bark of *C. paradisi* MACFADY (grapefruit). The structure **3** having a hydrogenated tropone ring system was elucidated for citropone-C.

Results and Discussion

The acetone extracts of *Citrus* plants were subjected successively to silica gel column, preparative centrifugally accelerated thin layer, and/or preparative thin layer chromatographies (TLC) to afford citropone-A (**1**) and -B (**2**) in 0.0008% and 0.0003% yields, respectively, from *C. grandis*. Citropone-A (**1**) was also obtained, in 0.003% yield, from *C. natsudaidai*. Citropone-C (**3**) was isolated in 0.0097, 0.00045, 0.0020, and 0.0010% yields from kiyomi × iyo, ogonkan × hyuganatsu, dunkan-grapefruit × hamlin and grapefruit, respectively.

Structure of Citropone-A (1)[5] Citropone-A (**1**) was obtained as red needles from acetone, mp 280—282 °C, and the molecular formula was determined as $C_{22}H_{19}NO_6$ by high resolution mass spectrometry (HR-MS). The ultraviolet (UV) absorption bands in methanol at $\lambda_{\max}$ 215, 272, and 410 nm revealed shifts typical of 1-hydroxy-9-acridones upon addition of $AlCl_3$ or NaOMe.[6] The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra (Table I)[7] coupled with the results of two-dimensional (2D) ^{1}H–^{13}C correlation spectroscopy (H–C COSY) showed signals assignable to one acetyl [δ_H 2.56 (3H, s); δ_C 28.42 (q) and 203.49 (s)], one *N*-methyl [δ 3.76 (3H, s); δ_C 47.00 (q)], and two carbonyls [δ_C 180.01 and 185.63] as well as an acetyl carbonyl, two intramolecularly hydrogen-bonded hydroxy groups [δ 14.10 and 16.57 (1H each, s)], two pairs of AB-type signals at δ_H 5.57 and 6.61 (each 1H, d, $J=9.8$ Hz) and at δ_H 6.67 and 7.97 (each 1H, d, $J=12.7$ Hz)], and a long-range coupled 1H signal at δ_H 6.35. Observations of ^{1}H- and ^{13}C-NMR signals assignable to two oxygen-linked tertiary methyls [δ_H 1.51 (6H, s); δ_C 26.92 ($2\times$q) and 77.00[8] (s)] and a disubstituted double-bond [δ 5.57 and 6.61 (each 1H, d, $J=9.8$ Hz)] suggested the presence of a dimethylpyran ring system in the molecule. In the ^{1}H detected heteronuclear multiple bond connectivity (HMBC) spectrum, the presence of a significant H–C three bond correlation between a hydrogen bonded proton at δ_H 14.10 and a carbon at δ_C 100.32 bearing a proton at δ_H 6.35 (H-2) suggested the angular orientation of the dimethylpyran ring. From these spectral data together

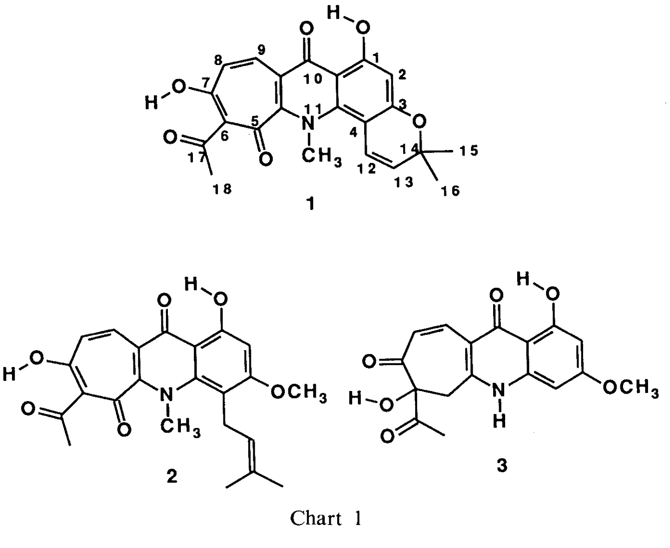

Chart 1

with other long-range H–C correlations in the HMBC spectrum (shown by arrows in Fig. 1), formula **1** was proposed as the structure of citropone-A.

A single-crystal X-ray analysis defined the complete structure of **1**. The crystal structure was solved by direct methods. Full-matrix least-squares refinement of atomic parameters converged at $R=0.043$ ($R_w=0.059$) over 2646 reflections with $I>3.0\sigma(I)$. Figure 2 shows a view of the structure with the atom numbering scheme. Final non-hydrogen atom coordinates are provided in Table IIa; hydrogen atom parameters and bonded distances are in Table IIb. In the main, bond lengths and angles involving the non-hydrogen atoms, listed in Table IIc, are in accord with expectations. Bond strain is, however, reflected in the significantly longer value of the C(12)–C(12a) distance at

TABLE I. ^{1}H- and ^{13}C-NMR Spectral Data for Citropone-A (**1**), -B (**2**), and -C (**3**)

	1	**2**	**3**
1-OH	14.10	14.19	14.94
			(br)
1	$\underline{164.26}$	$\underline{163.04}$	$\underline{164.48}$
2	6.35	6.47	6.62
	(d, $J=1.0$ Hz)		(1H, d, $J=1.5$ Hz)
	$\underline{100.32}$	$\underline{95.51}$	$\underline{98.23}$
3-OMe	—	3.93	3.63
			(3H, s)
	—	$\underline{56.23}$	$\underline{55.47}$
3	$\underline{161.88}$	$\underline{165.53}$	$\underline{165.14}$
4	—	—	6.82
			(1H, d, $J=1.5$ Hz)
	$\underline{101.94}$	$\underline{108.45}$	$\underline{92.07}$
4a	$\underline{143.88}$	$\underline{146.25}$	$\underline{142.27}$
5	—	—	3.51
			(1H, d, $J=14.7$ Hz)
	—	—	3.42
			(1H, d, $J=14.7$ Hz)
	$\underline{185.63}$	$\underline{185.56}$	$\underline{54.54}$
6-OH	—	—	$^{b)}$
6	$\underline{117.38}$	$\underline{117.25}$	$\underline{76.80}$
7	$\underline{176.68}$	$\underline{176.62}$	$\underline{199.40}$
7-OH	16.57	16.57	—
8	6.67	6.63	6.21
	(1H, d, $J=12.7$ Hz)	(1H, d, $J=12.7$ Hz)	(1H, d, $J=9.5$ Hz)
	$\underline{124.03}$	$\underline{123.50}^{a)}$	$\underline{118.44}$
9	7.97	7.93	8.23
	(1H, d, $J=12.7$ Hz)	(1H, d, $J=12.7$ Hz)	(1H, d, $J=9.5$ Hz)
	$\underline{133.42}$	$\underline{133.59}$	$\underline{139.24}$
9a	$^{b)}$	$\underline{116.36}$	$\underline{109.62}$
10	$\underline{180.01}$	$\underline{180.82}$	$\underline{178.83}$
10a	$\underline{108.59}$	$\underline{108.35}$	$\underline{108.31}$
N-Me	3.76	3.67	—
	(3H, s)	(3H, s)	
	$\underline{47.00}$	$\underline{46.74}$	—
11a	$\underline{153.80}$	$\underline{154.90}$	$\underline{158.00}$
12	6.61	3.45	—
	(1H, d, $J=9.8$ Hz)	(2H, d, $J=4.9$ Hz)	
	$\underline{120.51}$	$\underline{26.64}$	—
13	$\underline{5.57}$	$\underline{5.25}$	—
	(1H, d, $J=9.8$ Hz)	(1H, t, $J=4.9$ Hz)	
	$\underline{124.67}$	$\underline{123.36}^{a)}$	—
14	$\underline{77.00}^{c)}$	$\underline{132.56}$	—
15	1.51	1.75	—
	(3H, s)	(3H, s)	
	$\underline{26.92}$	$\underline{18.13}$	—
16	1.51	1.74	—
	(3H, s)	(3H, s)	
	$\underline{26.92}$	$\underline{25.61}$	—
17	$\underline{203.49}$	$\underline{203.65}$	$\underline{204.34}$
18	2.56	2.55	2.08
	(3H, s)	(3H, s)	(3H, s)
	$\underline{28.42}$	$\underline{28.43}$	$\underline{31.67}$

All spectra were measured on a GX-400 spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz; JEOL). Spectra of **1** and **2** were recorded in $CDCl_3$, and that of **3** in pyridine-d_5. Chemical shifts (δ ppm) of ^{1}H and ^{13}C signals are shown by normal and underlined letters, respectively. All proton signals appeared as 1H singlets, unless otherwise stated. Assignments of ^{13}C-signals were established by HMBC and H–C COSY techniques. *a*) Interchangeable. *b*) Not identified. *c*) Overlapped with the solvent.

TABLE IIa. Non-hydrogen Atom Fractional Coordinates, with Estimated Standard Deviations in Parentheses

Atom	*x*	*y*	*z*
C(1)	0.3105 (2)	−0.1396 (2)	0.4197 (2)
C(2)	0.3493 (2)	−0.2345 (2)	0.4697 (2)
C(3)	0.2311 (2)	−0.3500 (2)	0.4750 (2)
O(4)	0.0874 (1)	−0.3158 (1)	0.5114 (1)
C(4a)	0.0439 (2)	−0.2307 (1)	0.4388 (2)
C(5)	−0.1090 (2)	−0.2300 (2)	0.4405 (2)
C(6)	−0.1615 (2)	−0.1444 (2)	0.3719 (2)
C(6a)	−0.0615 (2)	−0.0589 (1)	0.2977 (2)
C(7)	−0.1209 (2)	0.0270 (2)	0.2236 (2)
C(7a)	−0.0116 (2)	0.1244 (2)	0.1701 (2)
C(8)	−0.0750 (2)	0.2202 (2)	0.1215 (2)
C(9)	−0.0166 (2)	0.3109 (2)	0.0430 (2)
C(10)	0.1288 (2)	0.3377 (2)	−0.0173 (2)
C(11)	0.2557 (2)	0.2939 (1)	0.0210 (2)
C(12)	0.2641 (2)	0.2276 (1)	0.1498 (2)
C(12a)	0.1393 (2)	0.1205 (1)	0.1766 (2)
N(13)	0.1897 (1)	0.0261 (1)	0.2265 (2)
C(13a)	0.0949 (2)	−0.0593 (1)	0.3008 (2)
C(13b)	0.1515 (2)	−0.1434 (1)	0.3772 (2)
C(14)	0.2671 (2)	−0.4156 (2)	0.6041 (2)
C(15)	0.2024 (2)	−0.4367 (2)	0.3251 (2)
O(16)	−0.3093 (1)	−0.1428 (1)	0.3737 (2)
O(17)	−0.2613 (1)	0.0216 (1)	0.2086 (2)
O(18)	0.1372 (2)	0.4166 (1)	−0.1142 (2)
C(19)	0.3950 (2)	0.3341 (2)	−0.0528 (2)
O(20)	0.4008 (2)	0.4075 (1)	−0.1454 (2)
C(21)	0.5328 ((2)	0.2851 (2)	−0.0293 (3)
O(22)	0.3758 (2)	0.2558 (1)	0.2388 (2)
C(23)	0.3324 (2)	0.0009 (2)	0.1699 (2)

TABLE IIb. Hydrogen Atom Fractional Coordinates,[a] Isotropic Thermal Parameters, and Bonded Distances, with Estimated Standard Deviations in Parentheses

Atom	*x*	*y*	*z*	*U* (Å^2)	*d* (Å)
H(1)	0.386 (2)	−0.061 (2)	0.421 (2)	0.010 (5)	0.99 (2)
H(2)	0.459 (3)	−0.230 (2)	0.507 (3)	0.023 (5)	1.02 (3)
H(5)	−0.178 (2)	−0.286 (2)	0.492 (2)	0.020 (5)	0.97 (2)
H(8)	−0.182 (2)	0.212 (2)	0.149 (2)	0.018 (5)	1.00 (2)
H(9)	−0.082 (2)	0.366 (2)	0.019 (3)	0.023 (6)	0.99 (2)
H(14A)	0.178 (3)	−0.482 (2)	0.614 (3)	0.037 (6)	0.99 (2)
H(14B)	0.365 (3)	−0.450 (2)	0.580 (3)	0.035 (6)	1.06 (3)
H(14C)	0.302 (3)	−0.356 (3)	0.697 (3)	0.057 (9)	0.99 (3)
H(15A)	0.123 (3)	−0.513 (2)	0.334 (2)	0.043 (8)	1.01 (2)
H(15B)	0.166 (3)	−0.391 (2)	0.243 (3)	0.029 (6)	1.04 (3)
H(15C)	0.295 (3)	−0.461 (2)	0.303 (3)	0.035 (6)	0.96 (3)
H(16)	−0.319 (3)	−0.076 (2)	0.314 (3)	0.051 (9)	1.00 (3)
H(18)	0.248 (3)	0.429 (2)	−0.149 (3)	0.042 (8)	1.05 (3)
H(21A)	0.506 (3)	0.204 (2)	0.001 (3)	0.043 (8)	0.97 (2)
H(21B)	0.588 (4)	0.284 (3)	−0.121 (4)	0.065 (10)	0.98 (4)
H(21C)	0.606 (4)	0.339 (3)	0.050 (4)	0.088 (13)	0.99 (3)
H(23A)	0.323 (3)	−0.085 (2)	0.167 (2)	0.013 (5)	0.95 (2)
H(23B)	0.338 (2)	0.025 (2)	0.069 (2)	0.016 (5)	0.98 (2)
H(23C)	0.426 (2)	0.046 (2)	0.239 (2)	0.020 (5)	1.02 (2)

a) Hydrogen atoms bear the same labels as the atoms to which they are bonded.

1.514(2) Å *vs.* 1.470(2) Å for the C(11)–C(12) bond. Endocyclic torsion angles characterising the solid-state conformation are reported in Table IId. Ring A, with small endocyclic torsion angles of 3.5° and −9.8° about the C(1)–C(2) and C(4a)–C(13b) bonds, respectively, is best described as a 1,3-diplanar form, while ring B, wherein the magnitudes of the torsion angles range from 0.2° to 6.4° (mean 3.2°), is fairly flat. Ring C is folded into a very shallow boat form to gain relief from non-bonded intramolecular overcrowding of the methyl group at the trigonal planar nitrogen centre and the hydrogen atom at C(1). Endocyclic

TABLE IIc. Interatomic Distances (Å) and Angles (°), with Estimated Standard Deviations in Parentheses

Bond lengths			
C(1)–C(2)	1.328 (3)	C(7a)–C(8)	1.442 (3)
C(1)–C(13b)	1.464 (3)	C(7a)–C(12a)	1.376 (3)
C(2)–C(3)	1.499 (3)	C(8)–C(9)	1.347 (3)
C(3)–O(4)	1.473 (2)	C(9)–C(10)	1.429 (3)
C(3)–C(14)	1.521 (3)	C(10)–C(11)	1.397 (3)
C(3)–C(15)	1.523 (3)	C(10)–O(18)	1.322 (3)
O(4)–C(4a)	1.351 (2)	C(11)–C(12)	1.470 (2)
C(4a)–C(5)	1.387 (3)	C(11)–C(19)	1.460 (3)
C(4a)–C(13b)	1.412 (2)	C(12)–C(12a)	1.514 (2)
C(5)–C(6)	1.374 (3)	C(12)–O(22)	1.217 (2)
C(6)–C(6a)	1.426 (2)	C(12a)–N(13)	1.371 (2)
C(6)–O(16)	1.343 (2)	N(13)–C(13a)	1.403 (2)
C(6a)–C(7)	1.434 (3)	N(13)–C(23)	1.478 (2)
C(6a)–C(13a)	1.416 (3)	C(13a)–C(13b)	1.416 (2)
C(7)–C(7a)	1.456 (3)	C(19)–O(20)	1.248 (3)
C(7)–O(17)	1.259 (2)	C(19)–C(21)	1.492 (3)
Bond angles			
C(2)–C(1)–C(13b)	120.8 (2)	C(8)–C(9)–C(10)	128.8 (2)
C(1)–C(2)–C(3)	120.1 (2)	C(9)–C(10)–C(11)	126.9 (2)
C(2)–C(3)–O(4)	108.3 (2)	C(9)–C(10)–O(18)	112.6 (2)
C(2)–C(3)–C(14)	112.1 (1)	C(11)–C(10)–O(18)	120.4 (2)
C(2)–C(3)–C(15)	112.8 (2)	C(10)–C(11)–C(12)	122.9 (2)
O(4)–C(3)–C(14)	104.1 (1)	C(10)–C(11)–C(19)	118.2 (2)
O(4)–C(3)–C(15)	107.9 (1)	C(12)–C(11)–C(19)	117.9 (2)
C(14)–C(3)–C(15)	111.2 (2)	C(11)–C(12)–C(12a)	122.7 (1)
C(3)–O(4)–C(4a)	118.4 (1)	C(11)–C(12)–O(22)	120.4 (1)
O(4)–C(4a)–C(5)	115.3 (1)	C(12a)–C(12)–O(22)	116.8 (2)
O(4)–C(4a)–C(13b)	121.0 (2)	C(7a)–C(12a)–C(12)	122.5 (2)
C(5)–C(4a)–C(13b)	123.5 (2)	C(7a)–C(12a)–N(13)	122.4 (1)
C(4a)–C(5)–C(6)	118.9 (2)	C(12)–C(12a)–N(13)	114.6 (1)
C(5)–C(6)–C(6a)	120.8 (2)	C(12a)–N(13)–C(13a)	121.1 (1)
C(5)–C(6)–O(16)	119.2 (2)	C(12a)–N(13)–C(23)	117.3 (2)
C(6a)–C(6)–O(16)	120.0 (2)	C(13a)–N(13)–C(23)	120.2 (1)
C(6)–C(6a)–C(7)	119.4 (2)	C(6a)–C(13a)–N(13)	117.6 (1)
C(6)–C(6a)–C(13a)	119.3 (2)	C(6a)–C(13a)–C(13b)	120.3 (1)
C(7)–C(6a)–C(13a)	121.3 (1)	N(13)–C(13a)–C(13b)	122.0 (2)
C(6a)–C(7)–C(7a)	117.2 (2)	C(1)–C(13b)–C(4a)	115.1 (2)
C(6a)–C(7)–O(17)	121.9 (2)	C(1)–C(13b)–C(13a)	127.3 (1)
C(7a)–C(7)–O(17)	120.8 (2)	C(4a)–C(13b)–C(13a)	117.0 (2)
C(7)–C(7a)–C(8)	114.8 (2)	C(11)–C(19)–O(20)	120.6 (2)
C(7)–C(7a)–C(12a)	118.7 (2)	C(11)–C(19)–C(21)	122.5 (2)
C(8)–C(7a)–C(12a)	126.4 (2)	O(20)–C(19)–C(21)	116.8 (2)
C(7a)–C(8)–C(9)	131.1 (2)		
Hydrogen-bonded distances (donor···acceptor)			
O(16)···O(17)	2.509 (2)	O(18)···O(20)	2.437 (3)

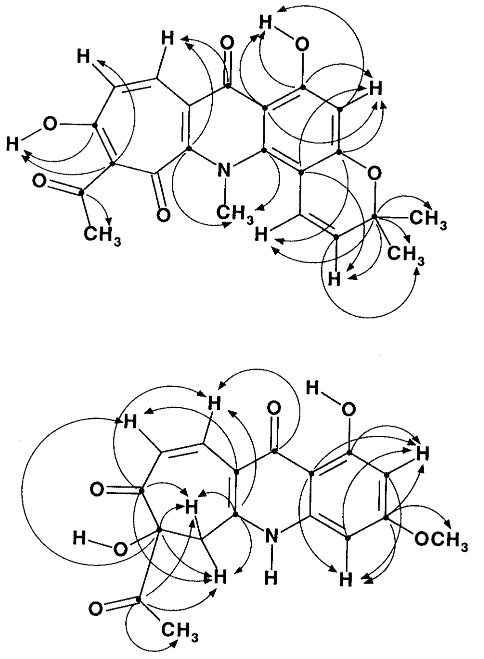

Fig. 1. C–H Correlations in HMBC Spectra of Citropone-A (**1**) and -C (**3**)

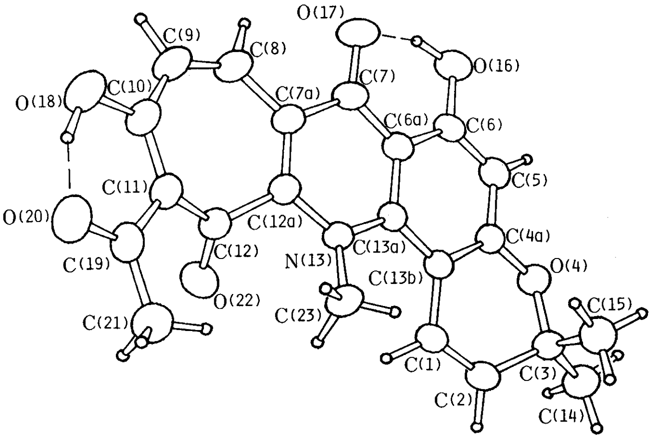

Fig. 2. ORTEP Diagram Showing the Solid-State Conformation of Citropone-A (**1**)

Small circles represent hydrogen atoms and the broken lines indicate intramolecular O–H···O hydrogen bonds.

TABLE IId. Torsion Angles[a] (°) Characterising the Ring Conformations in **1**

Ring A		Ring B		Ring C		Ring D	
C(13b)–C(1)–C(2)–C(3)	3.5 (3)	C(13b)–C(4a)–C(5)–C(6)	3.6 (3)	C(13a)–C(6a)–C(7)–C(7a)	−8.4 (3)	C(12a)–C(7a)–C(8)–C(9)	16.2 (3)
C(1)–C(2)–C(3)–O(4)	−34.3 (2)	C(4a)–C(5)–C(6)–C(6a)	1.1 (3)	C(6a)–C(7)–C(7a)–C(12a)	7.8 (3)	C(7a)–C(8)–C(9)–C(10)	0.7 (4)
C(2)–C(3)–O(4)–C(4a)	44.7 (2)	C(5)–C(6)–C(6a)–C(13a)	−2.7 (3)	C(7)–C(7a)–C(12a)–N(13)	2.4 (3)	C(8)–C(9)–C(10)–C(11)	−16.1 (3)
C(3)–O(4)–C(4a)–C(13b)	−24.3 (2)	C(6)–C(6a)–C(13a)–C(13b)	−0.2 (3)	C(7a)–C(12a)–N(13)–C(13a)	−12.5 (3)	C(9)–C(10)–C(11)–C(12)	−10.2 (3)
O(4)–C(4a)–C(13b)–C(1)	−9.8 (2)	C(6a)–C(13a)–C(13b)–C(4a)	4.5 (2)	C(12a)–N(13)–C(13a)–C(6a)	11.5 (2)	C(10)–C(11)–C(12)–C(12a)	49.3 (2)
C(4a)–C(13b)–C(1)–C(2)	20.4 (3)	C(13a)–C(13b)–C(4a)–C(5)	−6.4 (3)	N(13)–C(13a)–C(6a)–C(7)	−1.0 (2)	C(11)–C(12)–C(12a)–C(7a)	−47.5 (2)
						C(12)–C(12a)–C(7a)–C(8)	7.6 (3)

Estimated standard deviations are in parentheses. *a*) The torsion angle A–B–C–D is defined as positive if, when viewed along the B–C bond, atom A must be rotated clockwise to eclipse atom D.

torsion angles in ring D are related by an approximate mirror plane of symmetry passing through C(12) and the mid-point of the C(8)–C(9) bond, and this ring has an envelope-like conformation in which C(12) is the out-of-plane atom. The hydroxy groups at C(6) and C(10) are both involved in strong intramolecular hydrogen bonds to their respective adjacent carbonyl oxygen atoms [O(16)$\cdots$O(17) = 2.509(2) Å, O(18)$\cdots$O(20) = 2.437(3) Å].

Structure of Citropone-B (2)[5] Citropone-B (2) was isolated as orange needles from acetone, mp 192—194 °C. The HR-MS showed the molecular formula $C_{23}H_{23}NO_6$ for citropone-B. Close resemblance of the UV and ^{13}C-NMR spectra (Table I)[7] between **1** and **2** suggested that both alkaloids could have the same basic skeleton. The ^1H-NMR spectrum of **2** differs from that of **1** only by the presence of signals due to a methoxy (δ 3.93) and a prenyl [δ 1.74 (3H, s), 1.75 (3H, s), 3.45 (2H, d, $J = 4.9$ Hz), and 5.25 (1H, br t, $J = 4.9$ Hz)] group instead of signals ascribed to the dimethylpyran ring system in **1**; other signals assignable to an acetyl [δ 2.55 (3H, s)], *N*-methyl [δ_H 3.67 (3H, s)], two hydrogen-bonded hydroxy groups [δ_H 14.19 and 16.57 (each 1H, s)], a 1H singlet [δ_H 6.47 (H-2 or 4)] and AB-type signals [δ_H 6.63 and 7.93 (each 1H, d, $J = 12.7$ Hz), H-8 and -9] were observed, as in the spectrum of **1**. The appearance of mass fragment peaks at m/z 354 and 340, due to [$M^+ - \cdot CH{=}C(CH_3)_2$] and [$M^+ - \cdot CH_2CH{=}C(CH_3)_2$], respectively, also pointed to the presence of a prenyl moiety in the molecule. The location of the prenyl moiety at C-4 (not at C-2) was based on the following spectrometric observations: (a) In the HMBC spectrum of **2**, a correlation between the strongly hydrogen-bonded 1-hydroxy proton at δ_H 14.19 and the carbon at δ_C 95.51 bearing a proton at δ_H 6.47 was observed. (b) In the ^{13}C-NMR spectrum, the aryl carbon having a proton at δ_H 6.47 and the methylene carbon of the prenyl moiety appeared at δ_C 95.51 (d) and 26.64 (t), respectively.[10] On the basis of the foregoing spectral data, structure **2** was assigned for citropone-B.

Structure of Citropone-C (3) Citropone-C (3) was obtained as yellow prisms, mp 198—200 °C, $[\alpha]_D$ +7.14° (chloroform). The molecular formula was determined to be $C_{17}H_{15}NO_6$ by HR-MS. The ^1H- and ^{13}C-NMR spectrometric analyses (in pyridine-d_5) using the H–C COSY technique indicated the presence of a strongly hydrogen-bonded hydroxy (δ_H 14.94), an acetyl (δ_H 2.08; δ_C 31.67 and 204.34), an α,β-unsaturated carbonyl system [δ_H 6.21 and 8.23 (each 1H, d, $J = 9.5$ Hz), δ_C 199.40, 118.44 and 139.24], and an isolated methylene moiety [δ_H 3.42 and 3.51 (each 1H, d, $J = 14.7$ Hz); δ_C 54.54] along with a methoxy group (δ_H 3.63; δ_C 55.47), *meta*-located aromatic protons [δ_H 6.62 and 6.82 (each 1H, d, $J = 1.5$ Hz)], one more carbonyl (δ_C 178.83), and a fully substituted sp^3 carbon (δ_C 76.80). The connectivity of these structural elements was proposed as formula **3** based on the results of the HMBC experiments shown by arrows in Fig. 1, and the location of a hydroxy group at C-6 was also suggested. The observation of nuclear Overhauser effect (NOE) enhancements between the methoxy protons at δ_H 3.63 and protons at δ_H 6.62 (H-2) and 6.82 (H-4) also supported the substitution pattern on the aromatic ring. From these spectral data, we proposed the structure **3** for citropone-C except for the absolute stereochemistry.

Experimental

All melting points were measured on a micromelting point hot-stage apparatus (Yanagimoto). ^1H- and ^{13}C-NMR spectra were recorded on a GX-270 (JEOL) or GX-400 (JEOL) spectrometer in $CDCl_3$, unless otherwise stated. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. HMBC spectra were measured on $J = 8$ Hz. Electron impact (EI)-, fast-atom bombardment (FAB)-, and HR-MS were taken with an M-52, M-80 (Hitachi) or JMS-HX-110 (JEOL) mass spectrometer having a direct inlet system. UV spectra were recorded on a Jasco UVIDEC-610C double-beam spectrophotometer in methanol, infrared (IR) spectra on a Jasco IR-810 in $CHCl_3$, and optical rotation on a Jasco DIP-181 in $CHCl_3$. All TLC and preparative TLC procedures were done on Kieselgel 60 F_{254} (Merck).

Extraction and Separation The dried root bark (1.5 kg) of *C. natsudaidai* HAYATA was extracted with acetone at room temperature. The solvent was evaporated off *in vacuo* and the residue (119 g)[2b] was subjected to silica gel column chromatography eluted with benzene, CH_2Cl_2, acetone, and methanol, successively. The benzene eluate was subjected to additional silica gel column chromatography eluted with a gradient mixture of benzene–acetone. The 2% acetone–benzene eluate was concentrated *in vacuo*, and the residue was purified by repeated preparative TLC (solvents: appropriate mixtures of benzene, isopropyl ether, acetone, and methanol) to give a crystalline compound from benzene solution; this was recrystallized from acetone to give citropone-A (**1**) as red needles (46.1 mg). Other *Citrus* plants were treated in an analogous manner to obtain citropone-A (**1**), -B (**2**), and/or -C (**3**). From the dried root bark (1.45 kg) of *C. grandis* OSBECK f. *buntan* HAYATA collected in Taiwan, citropone-A (**1**) and -B (**2**) were isolated in 0.00075 and 0.00034% yields, respectively. Citropone-C (**3**) was isolated from the dried roots (208 g) of several hybrid seedlings resulting from crosses of [*C. unshiu* MARC. (miyagawa-wase) × *C. sinensis* OSBEC. (trobita orange)] (kiyomi) × *C. iyo* HORT. *ex* TANAKA (iyo),[4b] those (800 g) of *C. flaviculpus* HORT. *ex* TANAKA (ogonkan) × *C. Tamurana* HORT. *ex* TAKAHASHI (hyuganatsu),[4c] and those (132 g) of *C. paradisi* MACFADY (dunkan-grapefruit) × *C. sinensis* Osbeck (hamlin), and from the dried stem bark (779 g) of *C. paradisi* MACFADY (grapefruit) in 0.0097, 0.00045, 0.0020, and 0.0010% yields, respectively. *Citrus* plants used in this work, except for *C. grandis* were grown in the orchard of Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries, Shimizu, Shizuoka.

Citropone-A (1) Red needles from acetone, mp 280—282 °C. HR-MS (EI): Calcd for $C_{22}H_{19}NO_6$: 393.1210. Found: 393.1203. UV λ_{max} nm (log ε): 215 (4.26), 230 inf (4.25), 272 (4.39), 287 inf (4.38), 330 sh (4.09), 410 (3.84); λ_{max} (MeOH + $AlCl_3$) nm: 210 (log ε 4.37), 248 (4.33), 284 sh (4.54), 292 (4.56), 340 sh (4.11), 440 (3.95); λ_{max} (MeOH + NaOMe) nm: 212 (log ε 4.49), 270 (4.47), 282 inf (4.46), 307 sh (4.48), 324 (4.49). IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1610, 1570. MS m/z (%): 393 (M^+, 44), 378 (100), 350 (27), 315 (29), 300 (33), 299 (53), 270 (28), and 255 (22).

Crystal Data for Citropone-A (1)[9] $C_{22}H_{19}NO_6$, $M = 393.40$, triclinic, $a = 9.052(1)$, $b = 11.292(1)$, $c = 8.965(1)$ Å, $\alpha = 98.10(1)$, $\beta = 92.32(1)$, $\gamma = 102.87(1)°$ (from 25 orientation reflections, $51° < \theta < 66°$), $U = 882.0(3)$ Å^3, $Z = 2$, $D_c = 1.481$ g cm^{-3}, μ(Cu-K_α radiation, $\lambda = 1.5418$ Å) = 8.6 cm^{-1}. Space group $P1(C_1^1)$ or $P\bar{1}(C_i^1)$ from Laue symmetry; shown to be the latter by structure solution and refinement. Crystal dimensions: 0.04 × 0.06 × 0.70 mm.

Crystallographic Measurements Preliminary unit-cell parameters and space group information were derived from oscillation, Weissenberg, and precession photographs. One hemisphere of intensity data ($+h$, $\pm k$, $\pm l$, $\theta_{max} = 75°$; 3867 reflections) was recorded on an Enraf–Nonius CAD4 diffractometer [Cu-K_α radiation, graphite monochromator; ω–2θ scans, scan-width $(1.00 + 0.14 \tan\theta)°$]. The intensities of 4 reference reflections, remeasured every 2 h, showed no significant variation throughout the data collection. Following application of Lorentz and polarization corrections, equivalent reflections were averaged ($R_{merge} = 0.017$ on I) to yield 3627 reflections out of which those 2646 with $I > 3.0\sigma(I)$ were retained for the structure analysis and refinement.

Structure Analysis The crystal structure was solved by direct methods (MULTAN11/82) assuming at the outset that the centrosymmetric space group $P\bar{1}$ was the correct choice. Initial non-hydrogen atom coordinates were derived from an *E*-map. Several rounds of full-matrix least-squares adjustment of positional and thermal parameters (at first isotropic, then anisotropic) of these atoms were followed by evaluation of a difference Fourier synthesis which yielded hydrogen atom positions. With the inclusion of hydrogen atom positional and isotropic thermal parameters as variables in the subsequent least-squares iterations, and with the addition of an extinction correction (g) in the later cycles, the refinement converged at $R=0.043$ ($R_w=0.059$, $S=1.31$, $g=2.2\times10^{-6}$). Final non-hydrogen atom positional parameters are listed in Table IIa while hydrogen atom positional and thermal parameters are provided in Table IIb. Tables of final atomic positional and thermal parameters, bond lengths, bond angles, and torsion angles have been deposited at the Cambridge Crystallographic Data Centre.

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf–Nonius Structure Determination Package.[11] Neutral atom scattering factors and their anomalous dispersion corrections were taken from ref. 12. In the least-squares iterations, $\sum w\Delta^2[w=1/\sigma^2(|F_O|), \Delta=(|F_O|-|F_C|)]$ was minimized.

Citropone-B (2) Orange needles from acetone, mp 192—194 °C. HR-MS (EI): Calcd for $C_{23}H_{23}NO_6$: 409.1524. Found: 409.1538. UV λ_{max} nm (log ε): 217 (4.46), 230 sh (4.41), 265 (4.41), 309 (4.29), 330 sh (4.22), 407 (3.99); λ_{max} (MeOH + $AlCl_3$) nm (log ε): 213 (4.51), 232 inf (4.48), 280 (4.47), 310 (4.35), 340 sh (4.19), 430 (4.09); λ_{max} (MeOH + NaOMe) nm (log ε): 210 (4.61), 252 (4.36), 325 (4.48). IR ν_{max} cm^{-1}: 3200 (br), 1630, 1590. MS m/z (%): 409 (M^+, 96), 394 (100), 366 (19), 354 (49), 341 (37), 340 (45).

Citropone-C (3) Yellow prisms from acetone, mp 198—200 °C (dec.), $[\alpha]_D$ +7.14° ($c=0.056$, $CHCl_3$). HR-MS (EI): Calcd for $C_{17}H_{15}NO_6$: 329.0897. Found: 329.0889. UV λ_{max} nm: 206, 248, 283, 335, 341, 396. IR ν_{max}^{KBr} cm^{-1}: 3460 (br), 1715, 1665, 1645, 1540. ^{1}H-NMR (in acetone-d_6, 270 MHz) δ_H: 14.10 (1H, s, 1-OH), 7.90 (1H, d, $J=10$ Hz, H-9), 6.80 (1H, br s, H-4), 6.27 (1H, d, $J=2$ Hz, H-2), 6.00 (1H, d, $J=10$ Hz, H-8), 3.87 (3H, s, 3-OCH_3), 3.26 (1H, d, $J=15$ Hz, H-5), 3.16 (1H, d, $J=15$ Hz, H-5), 2.14 (3H, s, OAc). FAB-MS m/z: 330 ($M^+ + 1$). EI-MS m/z (%): 329 (M^+, 20), 314 (42), 274 (28), 207 (10), 185 (78), 115 (17), 98 (100).

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8) Overlapped with the solvent signal.

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