Citbismine-A, -B and -C, New Binary Acridone Alkaloids from *Citrus* Plants[1)]

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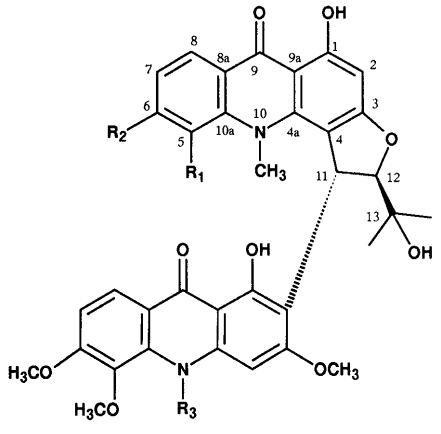
Three novel dimeric acridone alkaloids named citbismine-A (1), -B (3) and -C (5) have been isolated from the roots of Marsh grapefruit (*Citrus paradisi*) and Hirado-buntan (*C. grandis*). The structure of citbismine-A was elucidated as 1 by spectroscopic studies and single crystal X-ray analysis. Structure assignments of citbismine-B (3) and -C (5) were based on spectroscopic data including two dimensional (2D)-NMR (correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC)) experiments. The common structural characteristic is the presence of a C–C bond between the aromatic ring and the dihydrofuran ring of each acridone nucleus.

Key words citbismine-A; citbismine-B; citbismine-C; bisacridone alkaloid; *Citrus paradisi*; *Citrus grandis*

In our phytochemical studies on the constituents of *Citrus* plants, we have reported the isolation and structure elucidation of many new acridone alkaloids and coumarins. Unique constituents of this genus are dimeric compounds; acridone alkaloid and coumarin dimers (acrimarine-A—-N[2,3)] and neoacrimarine-A—-E[3-5)]) and dimeric coumarins (khelmarin-A, -B,[6)] -C, bisnorponcitrin, bishassanidin,[7)] bisosthenon,[8)] bisclausarin,[9)] nordenletin,[10)] citrumarin-A—-D,[11)] hassmarin,[12)] bisparasin,[13)] claudimerin-A,[14)] furobinordentatin and furobiclausarin[15)]). As a part of this program, we investigated the constituents of the roots of Marsh grapefruit (*Citrus paradisi* MACF.) and Hirado-buntan (*C. grandis* OSBECK f. Hirado) and isolated binary acridone alkaloids of a novel type, named citbismine-A (**1**), -B (**3**) and -C (**5**) for the first time from the genus *Citrus*. In this paper, we wish to present the isolation and structure elucidation of these compounds.

Citbismine-A (1) Citbismine-A (**1**) was isolated as yellow cubes, mp 335—336 °C, $[\alpha]_D$ ±0° (DMSO). The molecular formula $C_{35}H_{32}N_2O_{10}$ was established from the molecular ion peak at *m/z* 640.2068 in the high-resolution mass spectrum (HR-MS). The UV [λ_{max} (EtOH) 228 (sh), 265, 272, 298 (sh), 330, 384 nm] and IR [ν_{max} ($CHCl_3$) 3400, 1625, 1600, 1560 cm^{-1}] spectra indicated the presence of a 1-hydroxy-9-acridone nucleus.[16)] The ^{1}H-NMR spectrum showed two characteristic signals of chelated 1-hydroxyl groups [δ 15.29, 14.86 (each 1H, s, disappearing upon addition of D_2O)], suggesting the presence of two acridone nuclei. In the aromatic proton region, ABC-type [δ 7.56 (1H, d, J=7.9 Hz), 6.94 (1H, d, J=7.9 Hz), 7.04 (1H, t, J=7.9 Hz)], AB-type [δ 7.99, 7.17 (each 1H, d, J=9.2 Hz)], and two lone [δ 6.74, 6.18 (each 1H, s)] signals were observed. The two lowest signals of the ABC- and AB-type signals at δ 7.56 and 7.99 were considered to be deshielded by the 9- and 9′-carbonyl groups and could be assigned to H-8 and H-8′. Thus, the new compound was assumed to be a dimeric compound composed of 5-substituted-1-hydroxy-9-acridone and 5,6-disubstituted-1-hydroxy-9-acridone moieties. The presence of a 2-hydroxyisopropyl-substituted dihydrofuran moiety was indicated by the signals at δ 5.55, 4.37 (each 1H, d, J=4.9 Hz), 4.72 (1H, s, disappeared with D_2O), and 1.28, 1.23 (each 3H, s). The signals at δ 3.98, 3.85, 3.78, 3.41 (each 3H, s) in the ^{1}H- and δ 60.63, 56.31, 55.51, 44.34 in the ^{13}C-NMR spectra



	R_1	R_2	R_3
(1) citbismine-A	OH	H	H
(3) citbismine-B	OCH_3	OH	H
(5) citbismine-C	OCH_3	OH	CH_3

Chart 1

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Table 1. ^{1}H-NMR Chemical Shifts in DMSO-d_6

	1	3	5
1-OH	14.86	14.80	14.77
H-2	6.18	6.16	6.18
5-OH/OMe	9.95	2.72	2.71
H-6/OH	6.94 (d, 7.9)	a)	10.07
H-7	7.04 (t, 7.9)	6.76 (d, 8.8)	6.78 (d, 9.2)
H-8	7.56 (d, 7.9)	7.71 (d, 8.8)	7.73 (d, 9.2)
10-NMe	3.78	3.69	3.69
H-11	5.55 (d, 4.9)	5.54 (d, 5.4)	5.56 (d, 5.5)
H-12	4.37 (d, 4.9)	4.39 (d, 5.4)	4.41 (d, 5.5)
13-Me	1.23, 1.28	1.24, 1.28	1.26, 1.30
13-OH	4.72	4.72	4.71
1′-OH	15.29	15.37	15.65
3′-MeO	3.41	3.41	3.51
H-4′	6.74	6.74	6.39
5′-MeO	3.85	3.82	3.68
6′-MeO	3.98	3.97	3.98
H-7′	7.17 (d, 9.2)	7.18 (d, 8.8)	7.27 (d, 9.2)
H-8′	7.99 (d, 9.2)	8.01 (d, 8.8)	8.15 (d, 9.2)
10′-NH/NMe	11.24	11.21	3.87

Coupling constants (J values) in parentheses are in Hz. *a*) Signals could not be detected.

Table 2. ^{13}C-NMR Chemical Shifts in DMSO-d_6

	1	3	5
1	164.44	164.29[a)]	164.75
2	90.84	90.98	90.97
3	169.31	168.92	168.86
4	106.81	107.54	107.37
4a	145.40	145.07	146.28
5	147.73	135.63	137.90
5-MeO		57.82	57.79
6	119.23	156.25	157.71
7	122.52	112.92	112.65
8	115.07	121.72	122.04
8a	123.50	115.94	116.48
9	180.64	180.09	180.09
9a	105.45	104.80	104.77
10-NMe	44.34	44.36	44.18
10a	135.80	141.48	141.41
11	37.74	37.45	37.35
12	96.67	96.54	96.46
13	70.82	70.75	70.67
13-Me	24.69	24.71	24.65
	25.87	25.96	25.89
1′	158.91	158.82	159.34
2′	109.01	108.45	109.39
3′	164.33	164.32[a)]	164.27
3′-MeO	55.51	55.60	55.86
4′	89.39	89.32	89.33
4′a	142.38	142.46	145.02
5′	134.32	134.24	135.60
5′-MeO	60.63	60.62	60.90
6′	155.10	155.17	155.75
6′-MeO	56.31	56.33	56.41
7′	108.39	108.78	108.94
8′	121.36	121.38	121.65
8′a	114.19	114.02	116.13
9′	179.97	179.89	179.49
9′a	102.82	103.07	103.88
10′-NMe			40.18
10′a	135.40	135.36	136.78

a) Assignments may be interchanged.

indicated the presence of three methoxyl and N-methyl groups. In the nuclear Overhauser effect (NOE) experiment, irradiation of the methoxyl signal at δ 3.98 induced 8% enhancement of the signal at δ 7.17 (H-7′), suggesting the location of this methoxyl group to be at C-6′. When the methoxyl signal at δ 3.85 was irradiated, no increment was observed at any aromatic proton, so the location of this methoxyl group was assigned to be C-5′. Irradiation of the remaining methoxyl signal at δ 3.41 induced a 10% increment of the signal at δ 6.74, suggesting the location of this group to be at C-3 or C-3′. Treatment of citbismine-A with ethereal diazomethane afforded an *O*-methyl ether (**2**), indicating the presence of one free phenolic hydroxyl group. Further information was obtained by heteronuclear multiple-bond correlation (HMBC) experiments. The assignments of carbons and protons were made on the basis of comparisons with known acridone alkaloids, as well as H–C shift correlation spectroscopy (COSY). As shown in Fig. 1, the N-methyl group correlates to C-10a, NH correlates to C-8′a and C-9′a, and H-4′ correlates to C-2′, C-4′a and C-9′a. From the above-mentioned results, citbismine-A was considered to have a dimeric structure composed of 1,3,5-trioxygenated-9-acridone and 1,3,5,6-tetraoxygenated-9-acridone. Because attempted determinations of the location and orientation of the dihydrofuran ring and of the linking position of two acridone nuclei by HMBC experiments were unfruitful, a definitive structure was established unequivocally by X-ray crystallographic analysis. A molecule of citbismine-A found in an asymmetric unit is displayed in Fig. 2. The fractional atomic coordinates, individual bond lengths and bond angles are listed in Tables 3, 4 and 5, respectively. The molecule is dimeric, with two acridone nuclei joined between C-11 of previously unknown furo[2,3-*c*]acridone and C-2 of natsucitrine-II.

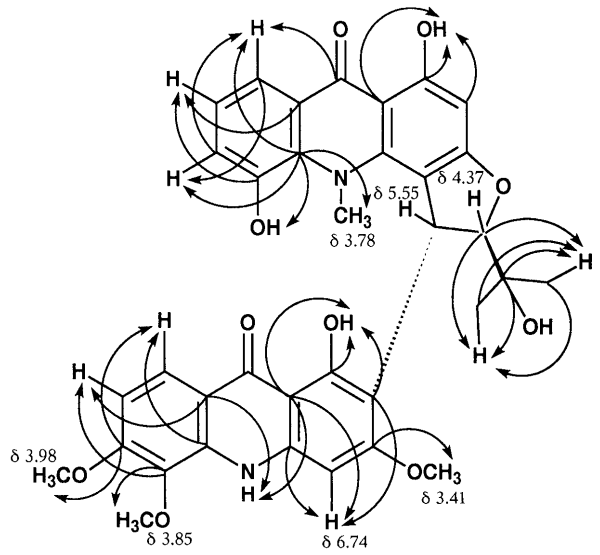

Fig. 1. C–H Long-Range Correlations in the HMBC Spectrum of Citbismine-A (**1**)

Citbismine-B (3) Citbismine-B was isolated as yellow cubes, mp 336—344 °C $[\alpha]_D$ ±0° ($CHCl_3$). Accurate mass measurement (peak matching) was obtained for the parent ion at m/z 671.2222 $(M+H)^+$ in the positive ion fast atom bombardment (FAB)-MS and provided the formula $C_{36}H_{34}N_2O_{11}$. The IR (3400, 1630, 1605, 1560 cm^{-1}) and UV [222 (sh), 265, 296 (sh), 330, 384 nm]

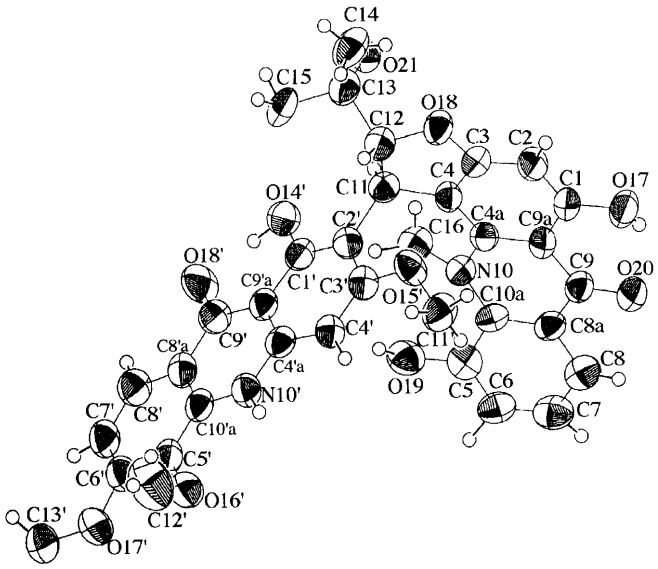

Fig. 2. Perspective View of Citbismine-A (**1**) with Atomic Numberings

spectra suggested the presence of a 1-hydroxy-9-acridone skeleton.[16] The dimeric structure was assumed from the molecular formula and the presence of two signals of chelated hydroxyl groups at δ 15.37 and 14.80 (each 1H, s) in the ^{1}H-NMR spectrum. Two *ortho*-coupled [δ 8.01, 7.18 (each 1H, d, J=8.8 Hz), 7.71, 6.76 (each 1H, d, J=8.8 Hz)] and two lone [δ 6.74, 6.16 (each 1H, s)] aromatic proton signals indicated the presence of two 1,3,5,6-tetraoxygenated-9-acridone nuclei. The existence of a 2-hydroxyisopropyl-3-substituted dihydrofuran moiety was assumed from the signals at δ 5.54 (1H, d, J=5.4 Hz), 4.39 (1H, d, J=5.4 Hz), 4.72 (1H, s), 1.24, 1.28 (each 3H, s) in the ^{1}H-NMR spectrum and the fragment ion at m/z 611, which arose by the loss of a C_3H_7O unit from the molecular ion. The presence of one N-methyl and four methoxyl groups was apparent from the signals at δ 3.69, 3.97, 3.82, 3.41 and 2.72 in the ^{1}H- and δ 44.36, 60.62, 57.82, 56.33 and 55.60 in the ^{13}C-NMR spectra. The location of these groups was deduced from NOE experiments. Irradiation of the methoxyl signal at δ 3.97 showed a 17% increment of the signal at δ 7.18 (H-7'), thus indicating this group to be at C-6'. When the methoxyl signal at δ 3.41 was irradiated, a 16% increment was observed in the signal at δ 6.74, suggesting this group to be located at C-3 or C-3'. Irradiation of the methoxyl signals at δ 3.82 and 2.72 showed no increment of any aromatic proton, indicating the location of these two groups to be C-5 or C-5'. On irradiation of the signal at δ 3.69, a 12% increment was observed in the signal at δ 5.54, and thus the N-methyl group should be located at N-10 and the angular orientation of the dihydrofuran moiety was established. Further information was obtained by HMBC experiments and the results are shown by arrows in Fig. 3. Important long-range correlations were as follows: the proton signal at δ 5.54 (H-11) correlates to C-3, C-4, C-12, C-1' and C-3'; the proton signal at δ 6.74 (H-4') correlates to C-2', C-3', C-4'a and C-9'a. Therefore, the two acridone units of citbismine-B were linked at C-11 of the dihydrofuran moiety and C-2' of the other acridone moiety. To confirm

Table 3. Fractional Atomic Coordinates and Their Estimated Standard Deviations in Parentheses

Atom	x	y	z	U (iso)
C9a	−0.1933 (5)	0.2176 (5)	−0.0213 (3)	0.052
C3'	−0.1953 (5)	0.4609 (5)	0.1537 (3)	0.055
C8'a	0.0663 (5)	0.4304 (5)	0.4021 (3)	0.059
N10'	0.0270 (4)	0.5749 (4)	0.2747 (3)	0.059
C2	−0.4297 (5)	0.2996 (6)	−0.0232 (3)	0.064
C10'a	0.0891 (5)	0.5415 (5)	0.3463 (3)	0.056
C3	−0.4229 (5)	0.2943 (5)	0.0555 (3)	0.054
C9'a	−0.0885 (5)	0.3919 (5)	0.3064 (3)	0.055
N10	−0.0756 (4)	0.1530 (4)	0.1045 (2)	0.049
C4'a	−0.0568 (5)	0.5010 (5)	0.2517 (3)	0.055
C10a	0.0472 (5)	0.1338 (5)	0.0632 (3)	0.051
C5'	0.1787 (6)	0.6189 (6)	0.3642 (3)	0.064
C2'	−0.2339 (5)	0.3529 (5)	0.2069 (3)	0.053
C11	−0.3362 (5)	0.2766 (5)	0.1819 (3)	0.051
C8a	0.0528 (5)	0.1445 (5)	−0.0190 (3)	0.054
C9'	−0.0282 (6)	0.3518 (5)	0.3825 (3)	0.063
C6'	0.2462 (6)	0.5824 (6)	0.4365 (3)	0.070
C9	−0.0714 (5)	0.1817 (5)	−0.0645 (3)	0.056
O1'	−0.2083 (4)	0.2117 (3)	0.3342 (2)	0.066
C4'	−0.1093 (5)	0.5359 (5)	0.1759 (3)	0.055
C1'	−0.1789 (5)	0.3184 (5)	0.2804 (3)	0.056
O1	−0.3209 (4)	0.2714 (4)	−0.1418 (2)	0.081
C4a	−0.1917 (5)	0.2048 (4)	0.0619 (3)	0.047
O3	0.1723 (4)	0.1131 (4)	0.1821 (2)	0.075
O3'	0.1973 (4)	0.7269 (4)	0.3085 (2)	0.079
O5'	−0.0551 (5)	0.2535 (4)	0.4322 (2)	0.084
C4	−0.3079 (5)	0.2493 (4)	0.1015 (3)	0.046
O2	−0.5273 (3)	0.3409 (4)	0.0965 (2)	0.066
O4	−0.0684 (4)	0.1919 (4)	−0.1387 (2)	0.073
O2'	−0.2565 (4)	0.4906 (3)	0.0830 (2)	0.064
C1	−0.3169 (5)	0.2610 (5)	−0.0625 (3)	0.058
O4'	0.3355 (4)	0.6634 (5)	0.4453 (2)	0.090
C8	0.1757 (6)	0.1217 (5)	−0.0600 (4)	0.067
C7'	0.2221 (6)	0.4738 (6)	0.4904 (3)	0.074
C5	0.1751 (6)	0.1091 (5)	0.1036 (3)	0.064
C8'	0.1333 (6)	0.3981 (6)	0.4743 (3)	0.069
C7	0.2944 (6)	0.0911 (6)	−0.0164 (4)	0.073
C6	0.2937 (6)	0.0873 (6)	0.0617 (4)	0.069
C12	−0.4807 (5)	0.3465 (5)	0.1728 (3)	0.059
C16	−0.0943 (6)	0.0795 (5)	0.1875 (3)	0.066
C14	−0.7279 (6)	0.3740 (7)	0.2139 (4)	0.084
C13	−0.5906 (5)	0.3017 (6)	0.2381 (3)	0.067
C15	−0.5449 (7)	0.3231 (8)	0.3153 (4)	0.094
C13'	0.3967 (7)	0.6439 (8)	0.5202 (4)	0.096
C11'	−0.1967 (6)	0.5753 (7)	0.0179 (3)	0.078
C12'	0.1326 (9)	0.8304 (8)	0.3300 (6)	0.122
O5	−0.6003 (4)	0.1752 (3)	0.2448 (2)	0.066(1)

the location of the free hydroxyl group, citbismine-B was treated with diazomethane to give an *O*-methylcitbismine-B (**4**). In the ^{1}H-NMR spectrum of **4**, one additional methoxyl signal was observed at δ 3.85. In the NOE experiment, irradiation of the newly generated methoxyl signal at δ 3.85 induced a 12% increment in the signal at δ 6.83 (H-7), showing the location of the newly formed methoxyl group to be C-6. The unusually high field (δ 2.72) resonance of the 5-methoxyl signal was explained on the basis of the shielding effect of the aromatic ring. Inspection of the molecular features of citbismine-B by use of a Dreiding model revealed that the 5-methoxyl group was situated within the field of shielding effect of the lower acridone nucleus. The relative stereochemistry of H-11 and H-12 was assigned as *trans* by comparison of their J-values (5.4 Hz) with those of citbismine-A (**1**) (4.9 Hz). From the

Table 4. Bond Distances (Å) with Estimated Standard Deviations in Parentheses

Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance
C9a	C9	1.455 (8)	C9a	C4a	1.408 (7)
C9a	C1	1.429 (8)	C3′	C2′	1.406 (8)
C3′	C4′	1.397 (8)	C3′	O2′	1.343 (7)
C8′a	C10′a	1.412 (8)	C8′a	C9′	1.465 (8)
C8′a	C8′	1.394 (8)	N10′	C10′a	1.362 (7)
N10′	C4′a	1.379 (7)	C2	C3	1.351 (8)
C2	C1	1.363 (8)	C10′a	C5′	1.409 (8)
C3	C4	1.402 (7)	C3	O2	1.358 (7)
C9′a	C4′a	1.408 (8)	C9′a	C9′	1.422 (8)
C9′a	C1′	1.445 (8)	N10	C10a	1.393 (7)
N10	C4a	1.396 (7)	N10	C16	1.473 (7)
C4′a	C4′	1.384 (7)	C10a	C8a	1.393 (8)
C10a	C5	1.445 (8)	C5′	C6′	1.398 (8)
C5′	O3′	1.378 (8)	C2′	C11	1.542 (7)
C2′	C1′	1.359 (8)	C11	C4	1.505 (7)
C11	C12	1.560 (8)	C8a	C9	1.462 (8)
C8a	C8	1.400 (8)	C9′	O5′	1.267 (8)
C6′	O4′	1.379 (8)	C6′	C7′	1.379 (9)
C9	O4	1.257 (7)	O1′	C1′	1.375 (7)
O1	C1	1.348 (7)	C4a	C4	1.418 (7)
O3	C5	1.366 (7)	O3′	C12′	1.418 (10)
O2	C12	1.440 (7)	O2′	C11′	1.421 (8)
O4′	C13′	1.416 (8)	C8	C7	1.395 (9)
C7′	C8′	1.376 (9)	C5	C6	1.372 (9)
C7	C6	1.340 (10)	C12	C13	1.537 (8)
C14	C13	1.538 (9)	C13	C15	1.517 (9)
C13	O5	1.438 (8)			

Table 5. Bond Angles (°) with Estimated Standard Deviations in Parentheses

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C9	C9a	C4a	120.2 (5)	C9	C9a	C1	120.2 (5)
C4a	C9a	C1	119.6 (5)	C2′	C3′	C4′	122.1 (5)
C2′	C3′	O15′	114.7 (5)	C4′	C3′	O15′	123.0 (5)
C10′a	C8′a	C9′	118.8 (5)	C10′a	C8′a	C8′	119.7 (5)
C9′	C8′a	C8′	121.5 (6)	C10′a	N10′	C4′a	122.2 (5)
C3	C2	C1	118.7 (5)	C8′a	C10′a	N10′	121.1 (5)
C8′a	C10′a	C5′	119.8 (5)	N10′	C10′a	C5′	119.1 (5)
C2	C3	C4	124.9 (5)	C2	C3	O18	121.7 (5)
C4	C3	O18	113.3 (5)	C4′a	C9′a	C9′	121.6 (5)
C4′a	C9′a	C1′	117.2 (5)	C9′	C9′a	C1′	121.1 (5)
C10a	N10	C4a	119.7 (4)	C10a	N10	C16	118.0 (5)
C4a	N10	C16	118.4 (4)	N10′	C4′a	C9′a	119.1 (5)
N10′	C4′a	C4′	119.6 (5)	C9′a	C4′a	C4′	121.3 (5)
N10	C10a	C8a	121.6 (5)	N10	C10a	C5	120.9 (5)
C8a	C10a	C5	117.4 (5)	C10′a	C5′	C6′	119.0 (6)
C10′a	C5′	O16′	117.9 (5)	C6′	C5′	O16′	123.1 (6)
C3′	C2′	C11	120.5 (5)	C3′	C2′	C1′	118.2 (5)
C11	C2′	C1′	121.3 (5)	C2′	C11	C4	116.3 (4)
C2′	C11	C12	110.3 (5)	C4	C11	C12	102.8 (4)
C10a	C8a	C9	119.8 (5)	C10a	C8a	C8	121.5 (5)
C9	C8a	C8	118.8 (5)	C8′a	C9′	C9′a	117.0 (5)
C8′a	C9′	O18′	120.7 (5)	C9′a	C9′	O18′	122.3 (6)
C5′	C6′	O17′	113.4 (6)	C5′	C6′	C7′	120.4 (6)
O17′	C6′	C7′	126.2 (6)	C9a	C9	C8a	116.8 (5)
C9a	C9	O20	122.2 (5)	C8a	C9	O20	120.7 (5)
C3′	C4′	C4′a	119.0 (5)	C9′a	C1′	C2′	122.1 (5)
C9′a	C1′	O14′	116.7 (5)	C2′	C1′	O14′	121.2 (5)
C9a	C4a	N10	120.6 (5)	C9a	C4a	C4	119.2 (5)
N10	C4a	C4	120.2 (5)	C5′	O16′	C12′	114.4 (6)
C3	C4	C11	107.6 (5)	C3	C4	C4a	116.8 (5)
C11	C4	C4a	135.1 (5)	C3	O18	C12	109.2 (4)
C3′	O15′	C11′	118.3 (5)	C9a	C1	C2	120.5 (5)
C9a	C1	O17	120.3 (5)	C2	C1	O17	119.0 (5)
C6′	O17′	C13′	118.5 (6)	C8a	C8	C7	118.1 (6)
C6′	C7′	C8′	121.3 (6)	C10a	C5	O19	117.7 (5)
C10a	C5	C6	119.5 (6)	O19	C5	C6	122.8 (6)
C8′a	C8′	C7′	119.9 (6)	C8	C7	C6	121.8 (6)
C5	C6	C7	121.5 (6)	C11	C12	O18	106.4 (5)
C11	C12	C13	117.3 (5)	O18	C12	C13	108.9 (5)
C12	C13	C14	109.1 (5)	C12	C13	C15	107.0 (5)
C12	C13	O21	107.8 (5)	C14	C13	C15	110.7 (6)
C14	C13	O21	110.3 (5)	C15	C13	O21	111.7 (5)

above-mentioned results, the structure of citbismine-B was concluded to be **3**.

Citbismine-C (5) This alkaloid was isolated as yellow cubes, mp 314—326 °C, $[\alpha]_D$ ±0° ($CHCl_3$). The HR-MS gave a molecular ion at *m/z* 684.2314, consistent with the molecular formula $C_{37}H_{36}N_2O_{11}$. The IR (3500, 1625, 1590 cm^{-1}) and UV [226, 264, 279 (sh), 335, 390 nm] spectra showed the presence of a 9-acridone skeleton.[16] The ^1H- and ^{13}C-NMR spectra (see Tables 1, 2) showed the presence of two chelated hydroxyl groups, two *ortho*-coupled and two lone aromatic protons, a 2-hydroxyisopropyl-substituted dihydrofuran moiety, four methoxyl and two N-methyl groups. These signal patterns were similar to those of citbismine-B (**3**) except for the presence of one additional N-methyl group signal [δ_H 3.87 (3H, s), δ_C 40.18 (q)]. The locations of methoxyl and N-methyl groups were determined on the basis of NOE experiments; irradiation of the signal at δ 3.98 induced a 14% increment of the signal at δ 7.27 (H-7′), and thus this group was assigned to C-6′. When the signals at δ 3.51 and 3.87 were irradiated, 14% and 16% increments were observed in the signal at δ 6.39 (H-4′). This result indicated the presence of a methoxyl group at C-3′ and an N-methyl group at N-10′. Irradiation of the signal at δ 3.69 induced a 12% increment of the signal at δ 5.56 (H-11), suggesting the presence of an N-methyl group at N-10 and the angular orientation of the dihydrofuran ring. The remaining two methoxyl signals at δ 2.71 and 3.68 gave no increment at any aromatic proton, so we concluded that the locations of these two methoxyl groups were at C-5 and C-5′, respectively. From the results mentioned above, the location of one phenolic hydroxyl group was concluded to be C-6. The relative stereochemistry of H-11 and H-12 was assigned as *trans* by comparisons of *J*-values with those of **1** and **3**.

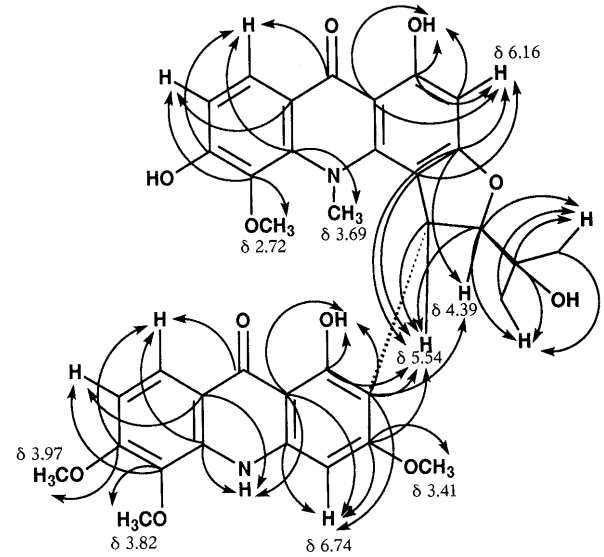

Fig. 3. C–H Long-Range Correlations in the HMBC Spectrum of Citbismine-B (**3**)

Lack of optical activity of citbismine-A, -B and -C suggests either that they are artifacts or that they are formed in the plant cells without the participation of enzymes.

The bisacridone alkaloids so far isolated from plant sources can be classified into two types: i) the two acridone units are linked by an ether bridge as in atalanine and ataline from *Atalania ceylanica*[17]; ii) the two acridone units are attached through a carbon–carbon bond as in glycobismine-A, -B and -C from *Glycosmis citrifolia* (WILD.) LINDL.[18] The common structural feature of citbismines reported here is the presence of a C–C bond connecting the aromatic ring and the dihydrofuran ring. Citbismine-A, -B and -C have a novel skeleton and could be classified into a third group. These are the first examples of bisacridone alkaloids isolated from *Citrus* plants.

Experimental

Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. UV spectra were measured on a Shimadzu UV-160A spectrometer and IR spectra were taken with a Shimadzu IR-435 spectrometer. EI- and HR-MS were measured with a JMS DX-303 (JEOL) spectrometer and HR FAB-MS with a JMS-HEX-110 (JEOL) spectrometer having a direct inlet system. NMR spectra were recorded on a JEOL 200FX or JEOL-GSX 500 spectrometer and chemical shifts are given on the δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. HMBC spectra were measured at $J=5$ and 8 Hz on the JEOL A-600 spectrometer. For column chromatography, Wakogel 60 was used. Preparative thin layer chromatography (PTLC) was carried out on precoated Merck Kieselgel plates.

Extraction and Isolation The plant material, both Marsh grapefruit and Hirado-buntan, was cultivated and collected at the Fruit Tree Research Station. The dried roots (1.2 kg) of Marsh grapefruit were extracted with acetone for 40 h under reflux (3 × 2 l). The acetone extract (138 g) was chromatographed over silica gel (1 kg) and the column was successively eluted with hexane, benzene, CH_2Cl_2, acetone–CH_2Cl_2, acetone and MeOH. The CH_2Cl_2 eluate (43.11 g) was submitted to silica gel column chromatography, centrifugal chromatography, and finally repeated PTLC [solvents: benzene–MeOH (9 : 1), $CHCl_3$–MeOH (19 : 1), acetone–hexane (1 : 1), benzene–AcOEt (7 : 3)] to give citbismine-A (**1**, 9.0 mg), citbismine-B (**3**, 9.2 mg) and citbismine-C (**5**, 2.0 mg). The dried roots (1.1 kg) of Hirado-buntan were extracted with acetone in the same manner as described for Marsh grapefruit. The acetone extract (101.2 g) was chromatographed over silica gel (1 kg) with toluene, CH_2Cl_2, acetone and MeOH. The CH_2Cl_2 eluate (29.394 g) was separated by silica gel column chromatography, centrifugal chromatography, and finally repeated PTLC using the same solvent systems as described above to furnish **1** (6.0 mg), **3** (26.0 mg) and **5** (3.3 mg).

Citbismine-A (1) Yellow cubes (from DMSO), mp 335—336 °C, $[\alpha]_D$ ± 0° ($c=0.1267$, DMSO). UV λ_{max} nm: 228 (sh), 265, 272, 298 (sh), 330, 384. IR ν_{max} cm^{-1}: 3400, 1625, 1600, 1560. HR-MS Calcd for $C_{35}H_{32}N_2O_{10}$ 640.2057. Found: 640.2068. EI-MS *m/z* (%): 640 (M^+, 53), 607 (22), 582 (35), 581 (42), 568 (36), 567 (base peak, 100), 339 (10), 301 (56), 281 (27). ^{1}H- and ^{13}C-NMR (DMSO-d_6) δ: see Tables 1 and 2.

***O*-Methylcitbismine-A (2)** Yellow amorphous powder. UV λ_{max} nm: 221, 228 (sh), 264, 289 (sh), 332, 398. IR ν_{max} cm^{-1}: 1630, 1605. EI-MS *m/z* (%): 654 (M^+, 5), 593 (3), 581 (10), 353 (32), 339 (18), 338 (40), 302 (21), 301 (base peak, 100), 295 (58), 286 (20), 256 (26), 252 (29), 243 (30), 240 (28). ^{1}H-NMR ($CDCl_3$) δ: 15.05 (1H, s), 14.70 (1H, s), 8.68 (1H, s), 8.14 (1H, d, $J=7.8$ Hz), 7.89 (1H, d, $J=8.3$ Hz), 7.11 (1H, t, $J=8.3$ Hz), 6.99 (1H, d, $J=8.3$ Hz), 6.98 (1H, d, $J=7.8$ Hz), 6.32 (1H, s), 5.96 (1H, s), 5.53 (1H, d, $J=5.4$ Hz), 4.51 (1H, d, $J=5.4$ Hz), 4.04 (6H, s), 3.73 (3H, s), 3.43 (3H, s), 3.24 (3H, s), 1.43 (3H, s), 1.41 (3H, s).

Citbismine-B (3) Yellow cubes (from acetone), mp 336—344 °C. $[\alpha]_D$ ±0° ($c=0.180$, $CHCl_3$). UV λ_{max} nm: 222 (sh), 265, 296 (sh), 330, 384. IR ν_{max} cm^{-1}: 3400, 1630, 1605, 1560. HR FAB-MS Calcd for $C_{36}H_{35}N_2O_{11}$ $[M+H]^+$ 671.2241. Found: 671.2222. EI-MS *m/z* (%): 670 (M^+, 19), 611 (26), 598 (15), 597 (35), 369 (11), 351 (38), 336 (24), 321 (34), 314 (15), 311 (16), 306 (31), 302 (28), 301 (base peak, 100), 287 (20), 286 (48), 272 (18), 268 (15), 258 (17), 256 (20), 243 (17), 228 (16). ^{1}H- and ^{13}C-NMR (DMSO-d_6) δ: see Tables 1 and 2.

***O*-Methylcitbismine-B (4)** Yellow prisms (from CH_2Cl_2), mp 297—300 °C (dec.). UV λ_{max} nm: 217, 263, 273 (sh), 334, 388. IR ν_{max} cm^{-1}: 1640, 1600. FAB-MS *m/z*: 685 $[M+H]^+$. EI-MS *m/z* (%): 684 (M^+, 1), 625 (3), 383 (20), 325 (28), 302 (30), 301 (base peak, 100), 287 (16), 286 (33), 272 (21), 267 (17), 243 (41), 240 (47). ^{1}H-NMR ($CDCl_3$) δ: 15.13 (1H, s), 14.77 (1H, s), 8.70 (1H, s), 8.12 (1H, d, $J=9.2$ Hz), 8.02 (1H, d, $J=8.8$ Hz), 6.98 (1H, d, $J=9.2$ Hz), 6.83 (1H, d, $J=8.8$ Hz), 6.31 (1H, s), 5.96 (1H, s), 5.54 (1H, d, $J=4.7$ Hz), 5.30 (1H, s), 4.48 (1H, d, $J=4.7$ Hz), 4.03 (3H, s), 4.02 (3H, s), 3.85 (3H, s), 3.78 (3H, s), 3.25 (3H, s), 2.95 (3H, s), 1.60 (3H, s), 1.42 (3H, s). NOE: irradiation at δ 4.03 (6′-MeO) – 6% enhancement at δ 6.98 (H-7′); irradiation at δ 3.85 (6-MeO) – 12% enhancement at δ 6.83 (H-7); irradiation at δ 3.78 (10-NMe) – 6% enhancement at δ 5.54 (H-11); irradiation at δ 3.25 (3′-MeO) – 9% enhancement at δ 5.96 (H-4′); irradiation at δ 8.70 (10-NH) – 15% enhancement at δ 5.96 (H-4′). ^{13}C-NMR ($CDCl_3$) δ: 96.65 (C-12), 72.60 (C-13), 61.10 (5′-MeO), 58.95 (6-MeO), 56.27 (5-MeO), 56.20 (6′-MeO), 55.42 (3′-MeO), 44.93 (10-NMe), 38.13 (C-11), 25.21, 24.31 (13-Me).

Citbismine-C (5) Yellow cubes (from acetone), mp 314—326 °C, $[\alpha]_D$ ±0° ($c=0.3133$, $CHCl_3$). UV λ_{max} nm: 226, 264, 279 (sh), 335, 390. IR ν_{max} cm^{-1}: 3500, 1625, 1590. HR-MS Calcd for $C_{37}H_{36}N_2O_{11}$ 684.2319. Found: 684.2314. EI-MS *m/z* (%): 684 (M^+, 14), 625 (33), 611 (27), 369 (26), 354 (26), 352 (16), 351 (44), 336 (28), 316 (25), 315 (100, base peak), 311 (16), 301 (35), 300 (69), 296 (14), 286 (19), 285 (19), 257 (14). ^{1}H- and ^{13}C-NMR (DMSO-d_6): see Tables 1 and 2.

Single-Crystal X-Ray Diffraction Analysis of Citbismine-A (1) A suitable crystal of citbismine-A (**1**) was obtained by recrystallization from acetone. The crystal data are as follows. Crystal system: triclinic, space group *P*-1, $a=9.837$ (3) Å, $b=11.466$ (3) Å, $c=17.277$ (3) Å; $\alpha=75.01$ (2) °, $\beta=86.46$ (2) °, $\gamma=84.58$ (2) °, $V=1872.6$ (9) Å^3, $Z=2$. The empirical formula is $C_{35}H_{32}N_2O_{10}$, $M=640$, and calculated density is 1.135 g/cm^3. Three-dimensional X-ray data were collected using graphite-monochromated CuK_α radiation ($\lambda=1.54178$) on a Mac science MXC 18 automatic four-circle diffractometer up to a maximum 2θ of 127°. Of 6938 reflections, 6105 were unique. The structure was solved by a direct method (MONTECARLO-MULTAN). As the crystal has two molecules in an unit cell, one half of the molecule is crystallographically independent. All non-hydrogen atoms were located on the initial E synthesis. Hydrogen atoms were located in the calculated positions. Block-diagonal least-squares refinement with anisotropic non-hydrogen atoms and isotropic hydrogens gave a conventional *R* factor of 0.1438. The anisotropic thermal parameters, the intramolecular torsion angles, and the intermolecular non-bonded distances, and the observed and calculated structure factors are available from one of the authors (S.T.) on request. The atomic coordinates, the bond lengths and angles, and the thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

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