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THE STRUCTURE OF ACRIMARINES,
 THE FIRST NATURALLY OCCURRING ACRIDONE-COUMARIN DIMERS¹⁾

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The structures of acrimarine-A (1), -B (2), and -C (3), novel
 acridone alkaloids carrying a coumarin unit, from Citrus funadoko
 Hort. ex. Y. Tanaka, have been elucidated by spectral experiments.

KEYWORDS—Citrus; Rutaceae; acridone; coumarin; acrimarine; dimer

The root of Citrus plants contain many kinds of coumarins.²⁾ We previously
 showed some plants of this genus also contained acridone alkaloids³⁾ as well as
 coumarins. In our continuing phytochemical studies of Citrus plants, we have
 isolated three novel acridone-coumarin dimers, named acrimarine-A (1), -B (2), and
 -C (3), from the root of C. funadoko Hort. ex. Y. Tanaka (Funadoko, Rutaceae).
 This is the first occurrence of acridone-coumarin dimers in nature, and we report
 here the structural elucidation of these novel alkaloids.

The acetone extract of the seedling roots of C. funadoko was fractionated by a
 combination of silica gel column and preparative thin layer chromatographies to
 give new alkaloids, acrimarines, along with acridones and coumarins.

Acrimarine-A (1), $C_{31}H_{29}NO_8$ [m/z 543.1874 (M^+ found); 543.1890, calcd] was
 obtained as a pale yellow oil, $[\alpha]_D -9.76^\circ$ ($c = 0.082$, $CHCl_3$) (yield: 0.0016% from
 dried root); UV λ_{max} (MeOH) nm: 205, 259 (sh.), 276, 295 (sh.), 332; IR ν_{max}
 ($CHCl_3$) cm^{-1} : 3400 (br), 1730, 1620, 1595. The NMR spectra⁴⁾ of 1 in H-H and H-C
 COSY revealed the presence of three methoxy, one N-methyl, and two allyl methyl (δ
 1.65, 1.81) groups (Table I). The observation of a strongly hydrogen-bonded
 proton signal at δ_H 15.08, a lower N-methyl proton signal at δ_H 3.95, and a carbo-
 nyl carbon signal at δ_C 180.4 in 1H - or ^{13}C -NMR spectra, together with UV absorp-
 tions and a strong IR band at ν_{max} 1620 cm^{-1} suggested the presence of 1-hydroxy-
 N-methyl-9-acridone system in this alkaloid. Additionally, a coumarin nucleus in
 the molecule was shown by the appearance of AB-type proton signals [δ_H 6.22 and
 7.64 ($J = 9.4$ Hz)], a lactone carbonyl carbon signal (δ_C 161.3), and IR bands at
 ν_{max} 1730 and 1595 cm^{-1} . In the aromatic proton region of the 1H -NMR spectrum,
ortho-coupled doublets [δ_H 6.94 and 8.17 ($J = 9.2$ Hz)] and three 1H singlets (δ_H
 6.35, 6.78, 7.62) were observed. The lower field doublet at δ_H 8.17 was charac-
 teristic to H-8 in the acridone,⁵⁾ and a singlet at δ_H 7.62 was assignable to H-5'
 in the coumarin.²⁾ The remaining NMR signals at δ_H 5.53 and 5.97 (each 1H doublet,

coupled to each other, $J = 7.8$ Hz) accompanied with two allyl methyls (δ_{H} 1.65, 1.81) indicated the presence of a prenyl group connected with two aryl moieties. The mass spectrum also supported this structural feature in showing two prominent ions at m/z 242 ($\text{C}_{15}\text{H}_{14}\text{O}_3$, 29%) and 301 ($\text{C}_{16}\text{H}_{15}\text{NO}_5$, 100%) corresponding to coumarin and acridone units, respectively, and together they comprise the molecular ion (m/z 543). In NOE experiments, irradiation of the doublet at δ_{H} 5.53 (H-1'') gave an enhancement of 7% of the singlet at δ_{H} 7.62 (H-5'), as well as the doublet at δ_{H} 5.97 (H-2''), and on irradiation of the doublet at δ_{H} 5.97

Table I. ^1H - and ^{13}C -NMR Spectra of Acrimarine-A (1), -B (2), and -C (3)

	1		2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1-OH	15.08	162.7	14.3	161.4	14.5	163.7
2		109.1		111.5	6.34	93.0
3		161.3		163.7		162.9
3-OMe			3.85	55.9	3.90	56.1
4	6.35	92.3	6.19	87.3		105.3
4a		146.1		140.9		139.4
5		136.9		133.6		132.4
5-OMe	3.75	61.3	4.01*	61.0	3.84	60.8
6		157.6		154.7*		152.4
6-OMe	4.00	56.3*	4.00*	55.7		
7	6.94 d	107.3	6.90 d	107.3	6.84 d	112.1
	(9.2)		(9.1)		(9.1)	
8	8.17 d	123.2	8.06 d	122.3	7.95 d	122.7
	(9.2)		(9.1)		(9.1)	
8a		117.5		115.2		113.9
9		180.4		181.0		181.3
9a		104.8		104.3		104.2
10a		138.4		134.9		135.6
N-Me	3.95	40.0				
N-H			8.45		9.05	

	1		2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2'		161.3		161.9		161.5
3'	6.22 d	113.0	6.20 d	112.2	6.23 d	113.1
	(9.4)		(9.4)		(9.4)	
4'	7.64 d	143.9	7.66 d	144.4	7.66 d	143.8
	(9.4)		(9.4)		(9.4)	
4'a		112.3		112.2		112.1
5'	7.62	128.6	7.56	128.3	7.48	127.3
6'		129.4		130.8		129.1
7'		159.8		160.9		160.7
7'-OMe	3.92	56.2*	3.75	56.2	3.76	56.1
8'	6.78	99.0	6.68	98.4	6.73	99.2
8'a		154.4		154.4*		154.7
1''	5.53 d	34.0	5.71	32.7	5.67	34.1
	(7.8)		(9.4)		(7.4)	
2''	5.97 d	123.0	5.96 d	124.4	5.76 d	123.1
	(7.8)		(9.4)		(7.4)	
3''		136.9		132.8		136.2
3''-Me	1.65	18.4	1.70	18.1	1.64	18.3
	1.81	25.8	1.79	26.0	1.82	25.9

Values are in ppm. Figures in parentheses are coupling constant (J) in Hz. All spectra were recorded in CDCl_3 . * Values with this superscript can be interchanged.

(H-2'') gave 6 and 7% enhancements of the signals at δ_H 5.53 (H-1'') and 7.62 (H-5'), respectively. These data indicated the location of the prenyl moiety in coumarin unit at C-6'. Further, irradiation of the N-methyl (δ_H 3.95) and O-methyl (δ_H 3.92 and 4.00) signals showed 8, 9, and 11% enhancements of the signals at δ_H 6.35 (H-4), 6.78 (H-8'), and 6.94 (H-7), respectively, indicating the location of the prenyl group in the acridone skeleton at C-2 (not C-4) and two methoxys at C-7' and C-6. No NOE enhancement at any aromatic protons was observed on irradiation of the methoxy protons at δ_H 3.75 (C-5). The above data were in accordance with the structure 1 for acrimarine-A. In agreement with this proposition, we applied the 1H - ^{13}C long-range COSY to 1. As shown in formula 1 the H-bonded proton signal at δ_H 15.08 showed long-range correlation with the carbon signals at δ_C 162.7 (C-1), 109.1 (C-2), and 104.8 (C-9a). Further, the proton signal at δ_H 5.53 (H-1'') was correlated with carbon signals at δ_C 162.7 (C-1), 109.1 (C-2), 161.3 (C-3), 128.6 (C-5'), and 129.4 (C-6'). Other long-range correlations observed were shown by arrows in formula 1. On the basis of these results, the structure of acrimarine-A was confirmed as 1.⁶⁾ Structural components of acrimarine-A (1) are the previously known acridone, grandisine-II (4)⁷⁾ and a coumarin, suberosin (6),⁸⁾ both were isolated from the same plant.⁹⁾

Acrimarine-B (2), yellow prisms from acetone, m.p. 288-290°C, $[\alpha]_D$ -7.14° (c = 0.056, $CHCl_3$) (yield: 0.0041%); UV λ_{max} nm: 204, 224, 252 (sh.), 274, 288 (sh.), 330; IR ν_{max} cm^{-1} : 3410, 1720, 1635, 1615, 1605. The MS showed the molecular ion at m/z 543 analyzing for $C_{31}H_{29}NO_8$ [m/z 543.1874 (M^+ found); 543.1891 calcd], isomeric with 1, and displayed important fragments at m/z 242 ($C_{15}H_{14}O_3$, 12%) and 301 ($C_{16}H_{15}NO_5$, 25%),¹⁰⁾ the same as those in 1. The 1H -NMR features were also similar to those of 1, and indicated a lower field singlet at δ_H 14.3, characteristic of an H-bonded OH attached to C-1 in the 9-acridone, ortho-coupled proton doublet [δ_H 6.90, 8.06, J = 9.1 Hz] assignable to H-7 and H-8, three singlets [δ_H 6.19 (H-4 or H-2), 7.56 (H-5'), and 6.68 (H-8')], two AB-type signals [δ_H 6.20, 7.66 (each 1H d, J = 9.4 Hz) and δ_H 5.71, 5.96 (each 1H d, J = 9.4 Hz)] due to H-3' and H-4' in the coumarin, and H-1'' and -2'' in a prenyl moiety, respectively, two allyl methyls (δ_H 1.70, 1.79), and four methoxy (δ_H 3.75, 3.85, 4.00, 4.01) groups. However, the ^{13}C -NMR spectrum lacked the signal owing to an N-methyl group, and the 1H -NMR spectrum showed a singlet at δ_H 8.45 assignable to N-H group. In NOE experiments, irradiation of the N-H proton signal (δ_H 8.45) induced 11% enhancements of the aromatic proton singlet at δ_H 6.19 (H-4) indicating the location of the prenylcoumarin moiety at C-2. Further irradiation of methoxy signals at δ_H 4.01 (and 4.00), 3.85, and 3.75 gave 12, 17, and 21% enhancements of the aromatic proton signals at δ_H 6.90 (d, H-7), 6.19 (s, H-4), and 6.68 (s, H-8'), respectively. The results of the 1H - ^{13}C long-range COSY spectrum (arrows in formula 2) established the structure of acrimarine-B as 2 corresponding to a dimer of suberosin (6) and des-N-methyl analogue of citpressine-II (5a)¹¹⁾ which also occurred in the same plant.⁹⁾

Acrimarine-C (3), a pale yellow oil, $[\alpha]_D$ -6.17° (c = 0.081, $CHCl_3$) (yield: 0.0023%), UV λ_{max} nm: 209, 256, 266, 284 (sh.), 328, IR ν_{max} cm^{-1} : 3380, 1725, 1640, 1620, 1610, gave a molecular ion at m/z 529 which analyzed for $C_{30}H_{27}NO_8$ [m/z 529.1711 (M^+ found); 529.1734 calcd], a difference of CH_2 compared with 1 or 2. The characteristic mass fragments appeared at m/z 242 ($C_{15}H_{14}O_3$, 37%) and 287 ($C_{15}H_{13}NO_5$, 100%) shifted 14 mass unit compared with those of 1 or 2. The 1H -NMR

signal pattern resembled that of 2 (Table I), except for a lack of one methoxy group, indicating the presence of suberosin (6) and citpressine (5) units in acrimarine-C, similar to 2. In NOE experiments, irradiation of methoxy protons at δ_H 3.76 and 3.90 resulted in 11 and 15% increases of the signals at δ_H 6.73 (H-8') and 6.34 (H-2 or 4), respectively. However, no NOE enhancement was observed on irradiation of a methoxy signal at δ_H 3.84. A characteristically different feature from 1 or 2 in the NOE experiments appeared on irradiation of the N-H proton (δ_H 9.05) and a doublet at δ_H 5.67 (H-1''). On irradiation of the N-H singlet (δ_H 9.05) gave 8 and 7% increases of a pair of doublets at δ_H 5.67 (H-1'') and 5.76 (H-2''), respectively. Inversely, 4 and 6% increases of the N-H singlet appeared in each case to be an irradiated pair of doublets at δ_H 5.67 (H-1'') and 5.76 (H-2''), respectively. These data indicated the location of suberosin (6) unit at C-4 in the acridone nucleus. In order to confirm this, LSPD and 1H - ^{13}C long-range COSY experiments were carried out. As shown in formula 3, an observation of correlation between the H-bonded proton at δ_H 14.5 and carbon at δ_C 93.0 bearing a proton with δ_H 6.34 strongly supported the location of suberosin (6) unit at C-4 as 3. In LSPD experiment, the double-doublet at δ_C 93.0 collapsed to a doublet on irradiation of the H-bonded proton at δ_H 14.5. Other results of the 1H - ^{13}C long-range COSY (arrows in formula 3) also supported the structure 3⁶⁾ of acrimarine-C corresponding to the dimer between suberosin (6), a common structural component of acrimarines, and the des-N-methylated analogue of citpressine-I (5b).¹¹⁾

Since the first isolation of acridone alkaloids from natural sources, many monomeric and binary acridones have been found.¹²⁾ However, the isolation of the acrimarines represents the first example of acridone-coumarin dimers from natural sources.

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