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CONSTITUENTS OF *CITRUS DEPRESSA (RUTACEAE)*. CHARACTERIZATIONS OF FIVE NEW ACRIDONE ALKALOIDS.

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Abstract ------Five new acridone alkaloids, citracridone-I (1a), citracridone-II (1b), citpressine-I (2a), citpressine-II (2b), and prenylcitpressine (3) have been isolated from the root bark of *Citrus depressa* as well as a known acridone alkaloid 5.

The important chinese drug "Chen-pi" has been used for treating heartache, abdominal pain, diarrhea, tussis, vomiting, inappetence and as an expectorant[1]. The peel of various large amont *Citrus* species have been used as the source of Chen-pi in Taiwan, and one of them has been identified with the peel of *Citrus depressa* Hayata naturally growed in Orchid island[2]. This time, we collected the root bark of this plant in Orchid island and studied the constituents. This report describes the isolation and the structural elucidation of five new alkaloids named citracridone-I, -II, citpressine-I, -II, and prenylcitpressine.

Citracridone-I: orange plates, m.p. 275-278°, $C_{20}H_{19}NO_5$. The UV spectrum (λmax 209, 270, 282(sh.), 340 and 400 nm) showed the typical absorption associated with a 9-acridone nucleus[3-6], and bathochromic shifts were observed in the presence of $NaOCH_3$ or $AlCl_3$[6], indicating the presence of phenolic hydroxyl groups. The presence of two one-proton singlets at δ 14.52 and 9.33 which disappeared on D_2O in the PMR spectrum indicated that at least one of the OH-groups was hydrogen bonded. Other signals in the PMR spectrum of citracridone-I are shown in Table 1. Among those signals, one of two AB-type quartets at δ 6.63 and 5.61 (J=10Hz), and a six-proton singlet at δ 1.53 could be assigned to a dimethylchromen system. Observation of a long range coupling between the signals at δ 6.63 and 6.23 attributed to C-1' and C-2 protons, respectively indicated that the dimethylchromen ring located in ring C. Furthermore, the NOE experiment showed 9.5% enhancement of the signal at δ 6.63 on irradiation at the frequency corresponding to the N-methyl protons at δ 3.75. Consequently, dimethylchromen ring was assigned an angular orientation. In another AB-type signal at δ 7.00 and 8.01, attributable to the *ortho*-located protons each other, the lower field signal affected the deshielding of 9-carbonyl moiety could be assigned to C-8 proton. In addition, no NOE enhancement between C_7-H at δ 7.00 and the methoxyl at δ 3.91 suggested the location of a methoxyl and a hydroxyl group at C-5 and C-6, respectively. The above data were in excellent accord with the structure 1a for citracridone-I.

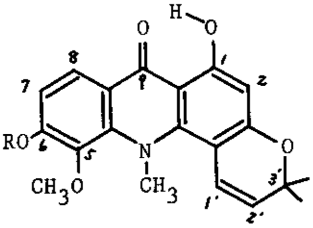
In agreement with this proposition, methylation of 1a with CH_2N_2 furnished a mono-O-methyl derivative of 1a, which was identified with citracridone-II (1b), m.p. 161-163°, $C_{21}H_{21}NO_5$, UV λmax 209, 268, 283, 341 and 405 nm, by comparisons with IR, UV, PMR and mass spectra and mixed m.p. Furthermore, catalytic hydrogenation of 1a led to yellow needles, m.p. 248-250°. Elemental analysis, IR, PMR and mass spectra of this product were in agreement with the structure 4 [7].

Citpressine-I, m.p. 183-185°, and citpressine-II, m.p. 168-170°, had the molecular formulae $C_{16}H_{15}NO_5$ and $C_{17}H_{17}NO_5$, respectively. Both showed the typical UV spectra[7] due to a 9-acridone nucleus[3-6]. The PMR spectra of both compounds showed the similar pattern except to an additional methoxyl signal in citpressine-II (Table 1). Treatment of citpressine-I with CH_2N_2 afforded a mono-O-methyl ether, which identified with citpressine-II. The presence of a hydroxyl group being still hydrogen bonded in citpressine-II was expected by the observation of IR (3430 cm^{-1}) and PMR spectra (1H singlet at δ 14.71). In the aromatic proton region of the PMR spectrum of citpressine-I, *meta*-coupled two protons signals at δ 6.25 and 6.51, and *ortho*-coupled two protons signals at δ 6.99 and 8.10 were observed. The lower *ortho*-coupled proton signal at δ 8.10 was characteristic to C_8-H in a 9-acridone system. The NOE experiment showed the signal at δ 6.51, one of *meta*-coupled protons, exhibited 14.8% and 20% enhancement on the irradiation at the frequency corresponding to the methoxyl proton signal at δ 3.97, and to the N-methyl proton signal at δ 4.10, respectively. And the same time, on the irradiation

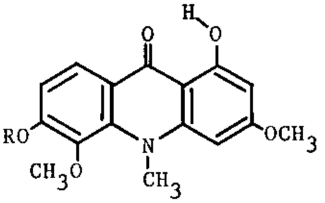
Table 1. PMR Spectra of Acridone Alkaloids

	1a	1b	2a	2b	3
1-H	14.52(1H,s)*	14.35(1H,s)*	14.85(1H,s)*	14.71(1H,s)*	14.40(1H,s)*
2-H	6.23(1H,d;1)	6.26(1H,s)	6.25(1H,d;2)	6.22(1H,d;2)	6.31(1H,s)
3-OCH_3			3.97(3H,s)	3.96(3H,s)	
4-H			6.51(1H,d;2)	6.46(1H,d;2)	
N-CH_3	3.75(3H,s)	3.74(3H,s)	4.10(3H,s)	4.04(3H,s)	3.64(3H,s)
5-OCH_3	3.91(3H,s)	3.90(3H,s)	3.83(3H,s)	3.84(3H,s)	3.92(3H,s)
6-OR	9.33(1H,s)*	4.01(3H,s)	2.83(1H,s)*	4.06(3H,s)	
7-H	7.00(1H,d;9)	7.01(1H,d;9)	6.99(1H,d;9)	7.16(1H,d;9)	6.95(1H,d;9)
8-H	8.01(1H,d;9)	8.13(1H,d;9)	8.10(1H,d;9)	8.12(1H,d;9)	7.94(1H,d;9)
1'-H	6.63(1H,dd;1,10)	6.62(1H,d;10)			3.58(2H,m)
2'-H	5.61(1H,d;10)	5.58(1H,d;10)			5.38(1H,m)
3'-$(CH_3)_2$	1.53(6H,s)	1.51(6H,s)			1.71(3H,s) 1.80(3H,s)

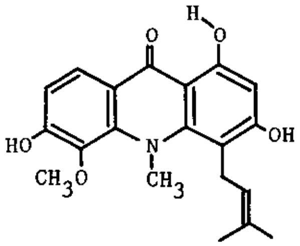
Run in acetone-d_6, except for 1d($CDCl_3$). Values are in ppm. Multiplicities are indicated by usual symbols: s, singlet; d, doublet; m, multiplet; dd, double doublet. Figures in parentheses are coupling constants in Hz. * Those signals disappeared on D_2O.



1a R=H
1b R=CH_3



2a R=H
2b R=CH_3



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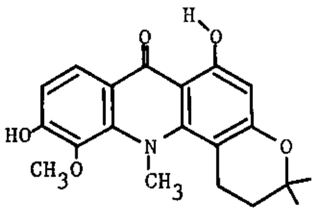

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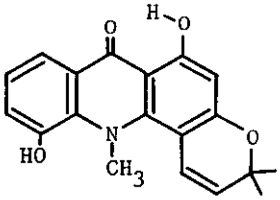

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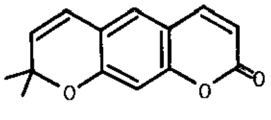

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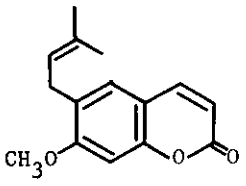

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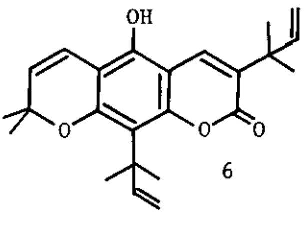

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to the methoxyl at δ 3.97, another *meta*-coupled proton signal at δ 6.25 were also increased in 22%. However, on the irradiation to the methoxyl at δ 3.83, no NOE enhancement was observed at any proton signals.

These chemical and spectral data led to the structure of citpressine-I and -II for 2a and 2b, respectively.

Prenylcitpressine: yellow needles, m.p. 160-162°, $C_{20}H_{21}NO_5$, UV λmax 222, 262, 269, 334 and 387 nm; IR νmax 3500, 3230, 1620, 1590 and 1555 cm^{-1}. The UV and IR spectra also indicated the characteristic absorption of 9-acridones[3-6]. The PMR studies revealed the presence of a strong intramolecular hydrogen bonded proton at δ 14.44 (disappeared on exchange with D_2O) assigned to C-1 hydroxyl group in acridone molecule. In addition, two hydroxyl protons at δ 9.24 and 6.96, which were also washed out with D_2O, were observed. Furthermore, the presence of an N-methyl, a methoxyl and a prenyl group in this alkaloid were suggested by the PMR spectrum (Table 1), and aromatic protons appeared as an *ortho*-coupled AB-type quartet and a sharp one-proton singlet. Cyclization of this compound with formic acid afforded yellow needles, m.p. 247-250°. The IR, PMR, UV and mass spectra of this product were superimposable with 4 which derived from citracridone-I (1a) as described above.

On the basis of these results, prenylcitpressine should be represented by formula 3.

A known acridone alkaloid 5 (no name)[6] was also isolated from the same plant. And as non-alkaloidal components, clarsarin (6)[8], suberosin (7)[9,10], and xanthyletin (8)[9,11] were isolated and identified with the authentic sample or their derivatives.

Chemical investigation of several species of the genus *Citrus* for their alkaloidal constituents indicated only the presence of simple alkylamines, quinolines and furoquinolines[12].

Our present study is the first example to show the presence of the acridone alkaloids in *Citrus* genus.

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7. Citpressine-I: UV λmax 220, 265(sh.), 271, 294(sh.), 332 and 384 nm. IR νmax(KBr) 3430, 1620, 1580, and 1560 cm^{-1}.
   Citpressine-II: UV λmax 220, 270, 300(sh.), 333 and 382 nm. IR νmax(KBr) 1650 and 1590 cm^{-1}.
   4 : PMR (acetone-d_6) δ 14.17(1H,s,1-OH), 6.08(1H,s,2-H), 3.72(3H,s,N-CH$_3$), 3.94(3H,s,O-CH$_3$), 6.98(1H,d,J=9Hz,7-H), 7.94(1H,d,J=9Hz,8-H), 2.95(2H,t, J=7Hz,1'-H), 1.86(2H,d,J=7Hz,2'-H), 1.45(6H,s,C(CH$_3$)$_2$). UV λmax 225, 266, 270, 336 and 382 nm. IR νmax(KBr) 3380, 1635, 1580 and 1552 cm^{-1}.
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