CONSTITUENTS OF CITRUS DEPRESSA (RUTACEAE). CHARACTERIZATIONS OF FIVE NEW ACRIDONE ALKALOIDS. Tian-Shung Wu and Hiroshi Furukawa\* Faculty of Pharmacy, Meijo University, Tempaku, Nagoya Chang-Sheng Kuoh Chia-Nan Junior College of Pharmacy, Tainan, Taiwan, R.O.C.

<u>Abstract</u> -----Five new acridone alkaloids, citracridone-I (la), citracridone-II (lb), 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<sup>1</sup>. The peel of various large amont *Citrus* species have been used as the source of <u>Chen-pi</u> in Taiwan, and one of them has been identified with the peel of *Citrus depressa* Hayata naturally growed in Orchid island<sup>2</sup>. 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.  $275-278^{\circ}$ ,  $C_{20}H_{19}NO_5$ . The UV spectrum ( $\lambda max 209, 270, 282(sh.)$ , 340 and 400 nm ) showed the typical absorption associated with a 9-acridone nucleus<sup>3-6</sup>, and bathochromic shifts were observed in the presence of NaOCH<sub>3</sub> or AlCl<sub>3</sub><sup>6</sup>, indicating the presence of phenolic hydroxyl groups. The presence of two one-proton singlets at  $\delta$  14.52 and 9.33 which disappeared on D<sub>2</sub>O 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 ABtype quartets at  $\delta$  6.63 and 5.61 (J=10Hz), and a six-proton singlet at  $\delta$  1.53 could be assigned to a dimethylchromen system. Observation of a long range coupling between the signals at  $\delta$  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  $\delta$  6.63 on irradiation at the frequency corresponding to the Nmethyl protons at  $\delta$  3.75. Consequently, dimethylchromen ring was assigned an angular orientation. In another AB-type signal at  $\delta$  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<sub>7</sub>-H at  $\delta$  7.00 and the methoxyl at  $\delta$  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 la for citracridone-I.

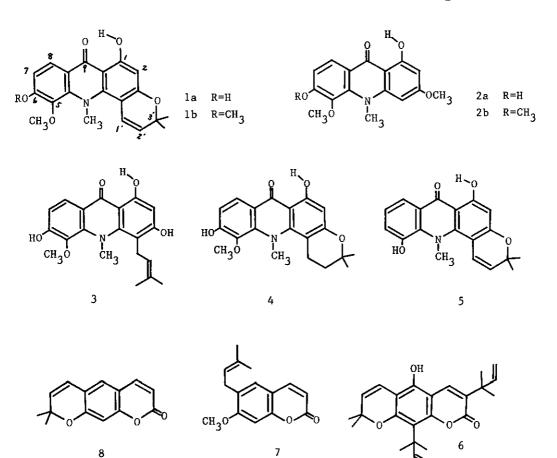
In agreement with this proposition, methylation of <u>la</u> with  $CH_2N_2$  furnished a mono-O-methyl derivative of <u>la</u>, which was identified with citracridone-II (1b), m.p. 161-163°,  $C_{21}H_{21}NO_5$ , UV  $\lambda$ max 209, 268, 283, 341 and 405 nm, by comparisons with IR, UV, PMR and mass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

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<sup>7</sup> due to a 9-acridone nucleus<sup>3-6</sup>. 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 CH2N2 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  $\text{cm}^{-1}$ ) and PMR spectra (1H singlet at & 14.71). In the aromatic proton region of the PMR spectrum of citpressine-I, meta-coupled two protons signals at  $\delta$  6.25 and 6.51, and ortho-coupled two protons signals at  $\delta$  6.99 and 8.10 were observed. The lower orthocoupled proton signal at  $\delta$  8.10 was characteristic to  $\rm C_8^{-H}$  in a 9-acridone system. The NOE experiment showed the signal at  $\delta$  6.51, one of <code>meta-coupled</code> protons, exhibited 14.8% and 20% enhancement on the irradiation at the frequency corresponding to the methoxyl proton signal at  $\delta$  3.97, and to the N-methyl proton signal at & 4.10, respectively. And the same time, on the irradiation

_	la	16	2a	2b	3
1-н	14.52(1H,s)*	14.35(1H,s)*	14.85(1H,s)*	14.71(1H,s)*	14.40(1H,s)*
2-н	6.23(1H,d;1)	6.26(1H,s)	6.25(1H,d;2)	6.22(1H,d;2)	6.31(1H,s)
3-0CH <sub>3</sub>			3.97(3H,s)	3.96(3H,s)	
4 <b>-</b> H			6.51(1H,d;2)	6.46(1H,d;2)	
N-CH3	3.75(3H,s)	3.74(3H,s)	4.10(3H,s)	4.04(3H,s)	3.64(3H,s)
50CH <sub>2</sub>	3.91(3H,s)	3.90(3H,s)	3.83(3H,s)	3.84(3H,s)	3.92(3H,s)
6-0R	9.33(1H,s) <sup>*</sup>	4.01(3H,s)	2.83(1H,s) <sup>*</sup>	4.06(3H,s)	
7 <b>-</b> 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 <b>'-</b> H	5.61(1H,d;10)	5.58(1H,d;10)			5.38(1H,m)
3'-( <sup>CH</sup> 3)2	1.53(6H,s)	1.51(6H,s)			1.71(3H,s) 1.80(3H,s)

Table 1. PMR Spectra of Acridone Alkaloids

Run in acetone- $d_6$ , except for <u>ld</u>(CDCl<sub>3</sub>). 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<sub>2</sub>O.



to the methoxyl at  $\delta$  3.97, another meta-coupled proton signal at  $\delta$  6.25 were also increased in 22%. However, on the irradiation to the methoxyl at  $\delta$  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  $\underline{2a}$  and  $\underline{2b}$ , respectively.

Prenylcitpressine: yellow needles, m.p.  $160-162^{\circ}$ ,  $C_{20}H_{21}NO_5$ , UV Amax 222, 262, 269, 334 and 387 nm; IR vmax 3500, 3230, 1620, 1590 and 1555 cm<sup>-1</sup>. The UV and IR spectra also indicated the characteristic absorption of 9-acridones<sup>3-6</sup>. The PMR studies revealed the presence of a strong intramolecular hydrogen bonded proton at  $\delta$  14.44 ( disappeared on exchange with  $D_20$  ) assigned to C-1 hydroxyl group in acridone molecule. In addition, two hydroxyl protons at  $\delta$  9.24 and 6.96, which were also washed out with  $D_20$ , 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<sup>°</sup>. The IR, PMR, UV and mass spectra of this product were superimposable with <u>4</u> 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 )<sup>6</sup> was also isolated from the same plant. And as non-alkaloidal components, clarsarin (6)<sup>8</sup>, suberosin (7)<sup>9,10</sup>, and xanthyletin (8)<sup>9,11</sup> 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<sup>12</sup>.

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

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- Citpressine\_I: UV λmax 220, 265(sh.), 271, 294(sh.), 332 and 384 nm. IR vmax(KBr) 3430, 1620, 1580, and 1560 cm<sup>-1</sup>. Citpressine-II: UV λmax 220, 270, 300(sh.), 333 and 382 nm. IR vmax(KBr) 1650 and 1590 cm<sup>-1</sup>. <u>4</u> : PMR (acetone-d<sub>6</sub>) δ 14.17(1H,s,1-OH), 6.08(1H,s,2-H), 3.72(3H,s,N-CH<sub>3</sub>), 3.94(3H,s,0-CH<sub>3</sub>), 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<sub>3</sub>)<sub>2</sub>). UV λmax 225, 266, 270, 336 and 382 nm. IR vmax(KBr) 3380, 1635, 1580 and 1552 cm<sup>-1</sup>.
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