

## ASSAMICADINE, A NEW PYRROLIZIDINE ALKALOID FROM *CROTALARIA ASSAMICA*

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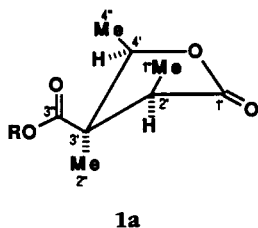
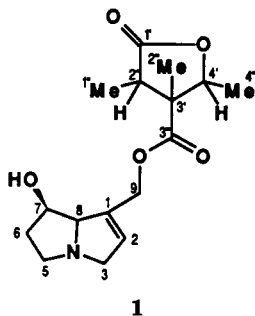
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**ABSTRACT.**—A minor alkaloid was isolated from *Crotalaria assamica*, and its structure was determined by spectroscopic methods as the 9-*O*-( $\gamma$ -lactone) of (2',3'-dimethyl-4'-hydroxypentane-2',3'-dicarboxyl)-retronecine [1]. The new alkaloid was named assamicadine.

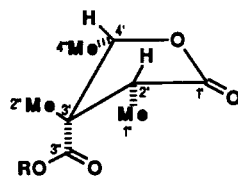
As we reported previously, monocrotaline has been isolated from *Crotalaria assamica* Benth. (Leguminosae), which occurs in the southern parts of China (1,2). The seeds of *C. assamica* yield about 3.5% of monocrotaline. During the further investigation of the seeds, another new pyrrolizidine alkaloid has been isolated which has been named assamicadine. This alkaloid occurs only in traces. Its structure **1** as the 9-*O*-( $\gamma$ -lactone) of (2',3'-dimethyl-4'-hydroxypentane-2',3'-dicarboxyl)-retronecine was determined by its physical and spectroscopic properties.

The ir spectrum of **1** showed characteristic signals for a free hydroxyl group at  $3373\text{ cm}^{-1}$ , a saturated ester at  $1736\text{ cm}^{-1}$ , and a  $\gamma$ -lactone structure at  $1778\text{ cm}^{-1}$ . The eims spectrum had a molecular ion peak  $[M]^+$  of  $m/z$  309 (4%), which can be correlated to the formula  $C_{16}H_{23}NO_5$ . Characteristic peaks appear at  $m/z$  266, 265, 256, 211, 155, 139, 138, 137, 136, 111, 94, 93, 80.

This fragmentation indicated the presence of the necine, retronecine, or one of its isomers. Alkaline saponification yielded a necine, whose spectroscopic data verify the structure to be that of retronecine.



**1a**



**1b**

The characteristic intensities of the ms fragments at  $m/z$  136, 137, and 138 support the presence of a C-9-mono-ester structure (3). The  $^1\text{H}$ -nmr chemical shift of H-7 at 4.46 ppm is an indication for the presence of a free hydroxyl group in position 7.

The DEPT spectrum of **1** gave the signals of five CH, four  $\text{CH}_2$  and three Me groups. The exact classification of the  $^1\text{H}$ -nmr signals was made by the  $^1\text{H}$ - $^1\text{H}$ -correlation spectrum. The 2D NOESY of assamicadine was used to determine the relative configuration of the molecule. The cross-signals in this spectrum showed an nOe effect indicating that H-2' is sterically near to the methylprotons of Me-1". The same is true for H-4' and Me-4". In addition, it was indicated that H-2' and Me-2" are on the same side of the ring plane of the lactone [**1a** and **1b**].

In the cd spectrum we observed a negative Cotton effect at 203 nm. The rule of Klyne and Beecham (5,6) concerning  $\gamma$ -lactones suggested that the  $\beta$  carbon lies under the ring plane of the  $\gamma$  lactone. The ester carbonyl group in po-

sition 3' is either pseudo-axial or pseudo-equatorial. H-2', H-4', and Me-2" are on the same side of the lactone ring system. The relative configuration of the lactone is either 2'S, 3'R, 4'R [**1a**] or 2'R, 3'S, 4'S [**1b**].

## EXPERIMENTAL

**APPARATUS.**—The melting points have been determined on a Kofler apparatus and are uncorrected. Optical rotation and cd spectra: J-20C, JSPCO, Japan. Ms spectra: JMS-D300, 70 eV,  $180^\circ$ .  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra: AM-400 Bruker, (400.1 MHz for  $^1\text{H}$ , 100.6 MHz for  $^{13}\text{C}$ ). The sample for all nmr experiments was 10 mg assamicadine in 0.4 ml  $\text{CDCl}_3$ ;  $\delta$  values are in ppm with TMS as internal standard.  $^1\text{H}$ - $^1\text{H}$ -correlation: AM-400 Bruker, COSY 45, conditions 256 exp./16 scans/1K; Relax-Delay = 0.4 sec; NOESY mixing time = 1000 msec.

**ISOLATION OF THE ALKALOIDS.**—Powdered seeds of *C. assamica* (3 kg) taken from the Hubei Institute of Botany, Academia Sinica, were exhaustively extracted with 96% MeOH. (Voucher specimens have been deposited in the Wuhan Botanical Institute, Academia Sinica.) The solvent was evaporated in vacuum, and the residue was dissolved in 2.5% HCl. The neutral compounds were eliminated by liquid-liquid extraction with  $\text{Et}_2\text{O}$  and  $\text{CH}_2\text{Cl}_2$ . The aqueous phase was basified with  $\text{NH}_3$  and again extracted with  $\text{CH}_2\text{Cl}_2$ . This purification procedure was re-

TABLE 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr Data of Assamicadine [**1**].

$^{13}\text{C}$			$^1\text{H}$		
Carbon	ppm	$J_{\text{C,H}}$ (Hz)	Proton	ppm	$J$ (Hz)
C-1	132.3 s		H-2	5.88 brs	
C-2	127.6 d	$J_1 = 167, J_2 = 7.0$	H-3	4.18 brd	15.7
C-3	61.8 t	$J_1 = 114$	H-3	3.57 brs	
C-5	53.7 t		H-5	3.52 m	
C-6	36.4 t	$J_1 = 134$	H-5	2.91 dd	18.4, 9.7
C-7	78.1 d		H-6	2.10 m	
C-8	81.2 d	$J_1 = 157, J_2 = 5.0$	H-7	4.46 brs	
C-9	61.8 t	$J_1 = 157, J_2 = 6.0$	H-8	4.46 brs	
C-1'	177.0 s		H-9	4.89 dd	14.8, 2.0
C-2'	46.1 d	$J_1 = 123, J_2 = 5.0$	H-9	4.68 dd	14.8, 2.0
C-3'	55.3 s		H-2'	2.61 q	7.0
C-4'	70.5 d	$J_1 = 155, J_2 = 5.0$	H-4'	4.30 q	6.5
C-1"	9.3 q	$J_1 = 124, J_2 = 2.0$	H-1"	1.20 d	7.0
C-2"	14.5 q	$J_1 = 157$	H-2"	1.38 s	
C-3"	171.0 s		H-4"	1.36 d	6.5
C-4"	18.5 q	$J_1 = 150, J_2 = 1.0$			

peated and yielded 105 g of pure monocrotaline. Alkaloids in the mother liquor were separated by preparative tlc [Si gel plates, 0.5 mm; eluent  $\text{CH}_2\text{Cl}_2$ -MeOH-NH<sub>3</sub> (25%) (85:14:1); detection Dragendorff reagent]. Removal of the Si gel and the extraction of the zone at  $R_f$  0.32 with  $\text{CH}_2\text{Cl}_2$  yielded monocrotaline, and the zone at  $R_f$  0.22 yielded assamicadine. The purification procedure was repeated twice, and 13 mg of assamicadine was isolated.

#### IDENTIFICATION OF THE ALKALOIDS.—

*Monocrotaline*.—Mp 200–201°;  $[\alpha]^{25}_D -55.5^\circ$  ( $c = 1$ ,  $\text{CHCl}_3$ ) [lit. (4) 202–203°,  $[\alpha]_D -55^\circ$  ( $\text{CHCl}_3$ );  $R_f = 0.32$  ( $\text{CH}_2\text{Cl}_2$ -MeOH-NH<sub>3</sub> (25%) (85:14:1)]; ir (KBr,  $\text{cm}^{-1}$ ) 3450 (-OH), 1735, 1710 (saturated and  $\alpha,\beta$ -unsaturated ester). The ms,  $^1\text{H}$ -nmr, and  $^{13}\text{C}$ -nmr data corresponded with those of an authentic sample (1).

*Assamicadine*.—Colorless, viscous material:  $[\alpha]^{19}_D -9.4^\circ$  ( $c = 0.4$ , MeOH); cd  $\lambda_2 = 203$  nm,  $\Delta\epsilon_2 = -4.4$ ;  $R_f = 0.22$ ; ir (KBr,  $\text{cm}^{-1}$ ) 3373 (-OH), 1778 ( $\gamma$ -lactone), 1736 (saturated ester); eims  $[\text{M}]^+$  309 (4.0), 266 (2.0), 265 (5.5), 256 (1.0), 211 (2.4), 155 (8.0), 139 (2.0), 138 (24), 137 (20), 136 (10), 111 (20), 94 (24), 93 (100), 80 (42); exact mass calcd for  $\text{C}_{16}\text{H}_{23}\text{NO}_5$ , 309.1570, found 309.1568;  $^1\text{H}$  and  $^{13}\text{C}$  nmr see

Table 1.

#### ACKNOWLEDGMENTS

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#### LITERATURE CITED

1. D.L. Cheng, S.B. Tu, A.A. Enti and E. Roeder, *Sci. Pharm.*, **54**, 351 (1986).
2. Group of *Crotalaria* Plant Res., *Chih Wu Hsueh Pao*, **16**, 380 (1974); *Chem. Abstr.*, **83**, 25052 (1975).
3. E. Pedersen and E. Larsen, *Org. Mass Spectrom.*, **4**, 249 (1970).
4. L.B. Bull, C.C.J. Culvenor, and A.T. Dick, "The Pyrrolizidine Alkaloids," North-Holland, Amsterdam, 1986, pp. 44, 279.
5. W. Klyne, P.M. Scopes, and A. Williams, *J. Chem. Soc.*, 7237 (1965).
6. A.F. Beecham, *Tetrahedron Lett.*, 3591 (1968).

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