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Two New Lactones Metabolized from Isoline by Rat Liver Microsomes

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Abstract: Two new metabolites, namely bisline lactone and isolinecic acid lactone, were isolated from the resultant incubates after a scale-up incubation of isoline with rat liver microsomes. Their structures were determined by spectroscopic data, especially those from 1D and 2D NMR experiments.

Keywords: Pyrrolizidine alkaloid, metabolite, isoline, bisline lactone, isolinecic acid lactone.

Isoline, a hepatotoxic retronecine-type pyrrolizidine alkaloid (PA), is present in several species of the genus *Ligularia* and *Senecio* (Compositae), for example, the antitussive traditional Chinese herb *Ligularia duciformis*[1]. Until now, few studies have been known about its metabolism and toxicity. In the recent investigation of *in vitro* metabolism of isoline by rat liver microsomes, we isolated two new non-pyrrolic metabolites, besides another two known, bisline **3** and retronecine **4**. We report here the structure elucidation of two new metabolites, namely bisline lactone **1** and isolinecic acid lactone **2**. A few evidences have shown that these metabolites are hydrolysis products by hepatic esterase(s). Further experiments related to their toxicity are now in progress in our laboratories.

Metabolite **1** was obtained as colourless oil. The UV spectrum showed maximum absorption at 217 nm. The HREIMS showed the molecular weight as 353.1842 (calcd. 353.1838), corresponding to the formula $C_{18}H_{27}NO_6$. EIMS gave rise to the molecular ion $[M]^+$ at *m/z* 353 and characteristic ion peaks at *m/z* 80, 93 and 120, which can be related to unsaturated necine (retronecine). The typical intensity of fragmentation at *m/z* 138 supported a 9-monoester structure. The IR spectrum showed characteristic signals for a saturated ester at 1734 cm^{-1}, and a δ-lactone structure at 1739 cm^{-1}. HMBC experiment showed that H-9 (δ_H 4.80) was correlated to C-11 (δ_C 170.5), gave the further evidence of the presence of a free hydroxyl group in position 7 and monoester structure in position 9. In the ^{13}C-NMR spectrum, carbon signals at δ_C 36.4 (C-6), 71.2

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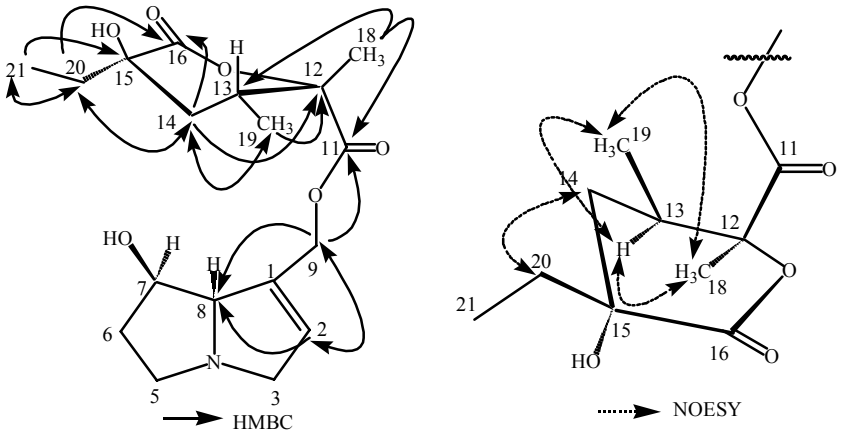
(C-7) and 77.9 (C-8) and two olefinic carbons at δ_C 132.6 (C-1) and 130.2 (C-2) represent the retronecine core of a pyrrolizidine alkaloid. Moreover, the quaternary carbon signals at δ_C 87.7 (C-12) and 73.5 (C-15) showed the large downfield shift and slightly upfield shift, respectively, from those of bisline **3**, and the ^{1}H and ^{13}C signals for 18-methyl group significantly shifted downfield for 0.46 ppm and 6.2 ppm, respectively, from that of bisline. These evidences clearly proved that the lactonization occurred between hydroxyl group on C-12 with carbonyl group on C-15 in necic acid moiety. The stereochemistry of metabolite **1** was determined by both NOESY and CD spectra. In NOESY, the cross signals between Me-18, H-13 and Me-19 indicated H-13, Me-18 and Me-19 were sterically close to each other, and Me-18 and H-13 were on the same side of the ring plane of the lactone. In CD spectrum a positive n→π* Cotton effect at 215 nm was observed. The rule of Klyne and Beecham concerning δ-lactone suggested that the C-14 lied up the ring plane of δ-lactone[2]. The relative stable conformation of the lactone could be determined as a half-chair type. Furthermore, because the interconversion between metabolite **1** and bisline has been confirmed in other experiments (data not shown), the absolute configurations of C-12, C-13 and C-15 should be S, R and R, respectively, the same as those for bisline[3, 4]. Therefore, metabolite **1** was identified as 9-*O*-(δ-lactone) of [(12S, 13R, 15R)-15-ethyl-15-hydroxy-12,13-dimethyl-17-oxotetrahydropyran-12,15-dicarboxylic acid] retronecine. This novel compound has not been reported and thus was named as bisline lactone (**Table 1, Figure 1**).

Figure 1 Structure and key HMBC and NOESY correlations for **1**



Metabolite **2** was yielded as a colourless needle, mp 113-115 °C (from $CHCl_3$/ Me_2CO). The EIMS did not show molecular ion peak but showed the fragments of loss of carboxyl group (-COOH) at *m/z* 171 and further loss of a CO group at *m/z* 143 (base peak). The fragmentation pattern was similar to that of isolinecic acid[5]. The FAB-MS showed a molecular ion peak $[M]^+$ at *m/z* 216.19, 18 mass unit (a water molecule) less than that of isolinecic acid ($C_{10}H_{16}O_5$). IR spectrum showed the possible δ-lactone absorption at 1735 cm^{-1} and a free carboxylic acid at 1694 cm^{-1}. The patterns of ^{1}H and ^{13}C NMR

spectrum were similar to isolinecic acid but the chemical shifts were different. Especially, the distinct difference of two oxyquaternary carbons could be observed at δ_C 72.9 for C-2 and 87.3 for C-5. In the ^{1}H NMR spectrum, the singlet at δ_H 3.09 (s, 1H) disappeared by adding deuterated water (D_2O), indicating to be a free hydroxyl proton. Moreover, this hydroxyl proton was shown long-range correlations with C-1 (δ_c 176.2) and C-3 (δ_c 33.4) in the HMBC spectrum and correlations with H-7 (δ_H 1.35) in the NOESY experiment. Thus, the free hydroxyl group was at C-2, and the hydroxyl group at C-5 formed the monolactone with carboxylic group at C-2. The relative configuration was also determined by NOESY spectrum. Similarly, the absolute configurations at C-2, C-3 and C-5 were tentatively assigned to be S, R and R, respectively, since spontaneous lactonization was shown to be possible in other study. From these findings, metabolite **2** was identified as (2S, 3R, 5R)-5-ethyl-2-hydroxy-2,

Figure 2 Structure and key HMBC and NOESY correlations for **2**

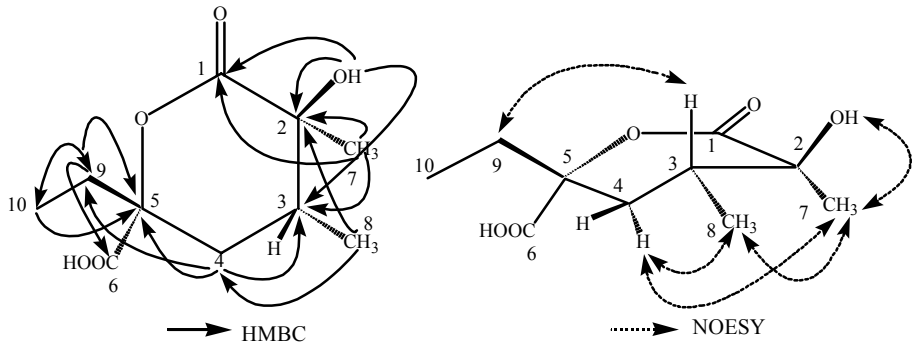


Table 1 ^{1}H and ^{13}C NMR spectral data of bisline lactone **1** and isolinecic acid lactone **2** (400/100MHz, $CDCl_3$, δ ppm)

C/H No.	**1**		**2**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		132.6 (s)		176.2 (s)
2	5.87 (br s)	130.2 (d)		72.9 (s)
3	3.92 (dt, 15.9, 2.2), 3.41(m)	62.9 (t)	2.35 (m)	33.4 (d)
4			2.20 (dd, 15.0, 4.2), 1.97 (m)	36.1 (t)
5	3.26 (td, 8.1, 1.7), 2.72 (m)	53.7 (t)		87.3 (s)
6	1.97 (m)	36.3 (t)		173.8 (s)
7	4.27 (br. s)	71.2 (d)	1.35 (s)	21.2 (q)
8	4.14 (br. s)	77.9 (d)	1.08 (d, 7.0)	14.1 (q)
9	4.80 (br. s)	62.8(t)	1.97 (m)	32.1 (t)
10			1.00 (t, 7.0)	8.0 (q)
11		170.5 (s)		
12		87.7 (s)		
13	2.39 (m)	34.2 (d)		
14	1.76 (dd, 14.6, 3.9), 1.63 (d, 14.6)	36.7 (t)		
15		73.5 (s)		
16		173.9 (s)		
18	1.68 (s)	23.6 (q)		
19	1.09 (d, 7.1)	16.1 (q)		
20	1.85 (q, 7.6)	32.5 (t)		
21	0.95 (t, 7.6)	7.3 (q)		
OH			3.09 s (Disappearing on D_2O exchange)	

3-dimethylhexanedioic acid-1-*O*-5 lactone. This new compound was named as Isolinecic acid lactone (**Table 1, Figure 2**).

Bisline lactone **1**. A colourless oil. $[\alpha]_D^{26}$ +39.2 (c 2.3, MeOH). UV λ_{max} (MeOH) nm: 217, 205. CD (MeOH):$\Delta\varepsilon_{215}$ +3.15, $\Delta\varepsilon_{203}$ +0.20. IR ν ($CHCl_3$) cm^{-1}: 3566, 2940, 2856, 1739, 1734, 1684, 1653, 1457, 1227, 1125. EI-MS *m/z*: 353 $[M]^+$, 309 (M^+-CO_2), 238 (309-$CH_3CH_2CHOCH_2$), 220, 196, 155, 138, 120, 111, 93, 80. ^{1}H-NMR and ^{13}C-NMR are listed in **Table 1**.

Isolinecic acid lactone **2**. A colourless needle. $[\alpha]_D^{30}$ +20 (c 0.4, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm: 207. IR ν ($CHCl_3$) cm^{-1}: 3516, 2930, 1735, 1694, 1599, 1131. FAB-MS *m/z*: 216.19 $[M]^+$, 238.15 $[M-1+Na]^+$. EI-MS *m/z*: 171$[M-CO_2H]^+$, 143 $[M-CO_2H-CO]^+$, 125, 100, 82-85. ^{1}H-NMR and ^{13}C- NMR are listed in **Table 1**.

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