HETEROCYCLES, Vol. 61, 2003, pp. 9 -12

Received, 10th March, 2003, Accepted, 8th April, 2003, Published online, 14th April, 2003

2-AMINO-5-HYDROXYHEXANOIC ACID AND ITS SUCCINYL DERIVATIVES AS THEIR LACTONE FORMS FROM KOBUTORISOU, AN ANTAGONIZING PLANT TO NEMATODE

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Abstract-Minute amounts of 2-acetylamino-5-hydroxyhexanoic acid (**1**) and its succinyl derivatives (**2a**) and (**2b**) as their lactones, and 2,3-diacetoxy-2-methyl-butanolide (**3**) were isolated from kobutorisou, an antagonizing plant to nematode, after Ac_2O-treatment of the anti-nematode fraction of extracts of kobutorisou.

It is well known that an area under continuous cultivation of a sole crop for several years frequently becomes rich with a specific nematode of such genera as *Meloidogyne* and *Heterodera* which live within their specific host crop, giving rise to serious effect on the yield of the crop. In order to counterplot against the increasing number of nematode, some sort of plant which is antagonistic to the nematode has been employed in the field; marigolds are well-known antagonizing flora which produces α-terthienyl possessing anti-nematode activity.[1]

Kobutorisou is a Japanese name of a plant developed for agricultural use against injury caused by nematode. The name of kobutorisou means a herb which gets rid of tubercle formation on the roots of crops caused by parasitic nematode. Kobutorisou is a cultivar species of a genus, *Crotalaria sessiliflora* , belonging to the *Leguminosae* family, and the seeds are commercially available. Genus *Crotalaria* is a rich source[2] of pyrrolizidine alkaloids possessing a retronecine skeleton as typified by monocrotalines I and II, partly attributed to the anti-nematode activity.[3,4] We are much interested in exploring the nematocid in kobutorisou. Here we report the isolation of minute amounts of δ-lactones (**1**), (**2a**) and (**2b**), and γ-lactone (**3**) from kobutorisou after Ac_2O-treatment of the active fraction obtained therefrom.

Before MeOH (2 L) was added to dried stems (2.7 kg) of kobutorisou (height: *ca.* 2.5 m) cultivated in the field, they were cut to *ca.* 10 cm long, and water (20 L) was added the next day. After storage for 2 weeks, the combined aq. solutions were evaporated to give crude extracts (250 g) with 90% anti-nematode activity at 2×10^3 ppm aq. solution. Partition experiments into organic solvents revealed that the active substance(s) was insoluble in acetone and soluble in MeOH and EtOH. After successive treatments with acetone, MeOH and then EtOH, the active EtOH soluble part was partly purified with Sepabeads gel and then Sephadex LH-20 gel chromatographies developed with water. The activity was observed in respective water effluents. The sequential separation procedures suggested that the active constituent was composed

of some sort of salt. The separation with usual ion exchange chromatography was unsuccessful due to disappearance of the original activity, and the active part was submitted to reaction with Ac_2O in pyridine at 50 °C for 2 days. The ether soluble part obtained therefrom was subjected to chromatographic purification under various conditions to isolate **1**, **2**, and **3** in addition to several kinds of sugars as their acetate.

The molecular formula of $C_8H_{13}NO_3$ for **1**, mp 126-128 °C; $[\alpha]_D^{27}$ +126.7° (c 0.5, $CHCl_3$) was estimated from HRMS spectrum, and was supported by the ^{13}C and ^1H NMR spectra (Table 1). Eight carbon atoms were clearly observed in the ^{13}C NMR spectra, in which two carbonyl and two methyl groups were detected, supporting the existence of lactone and amide moieties. IR (CCl_4) at 1730 cm^{-1} is consistent with this deduction. The detailed inspection of the ^1H NMR spectra ($CDCl_3$) including H-H COSY led to the final structure, possessing δ-lactone with chair conformation on which acetylamino and methyl groups are appended equatorially at the 2 and 5 positions, respectively. 2*S*-Configuration was deduced from the established *S* configuration of a usual amino acid such as L-lysine, a putative biogenetical precursor of **1**.

The compounds (**2a**) and (**2b**) were obtained as an inseparable mixture consisting of a single peak in the HPLC analysis under several conditions. The highest mass ion of the single peak showed at *m/z* 211 corresponding to $C_{10}H_{13}NO_4$. Irrespective of the presence of total 13 hydrogens in the molecular formula, the ^1H NMR spectrum showed fairly complicated peaks, some of which appeared independently at close positions in the ratio of 7:6. The detailed inspection of ^1H NMR spectra allows us to conclude the single peak in the HPLC consists of a 7:6 mixture of **2a** and **2b**, respectively. The HMBC study centered at δ 4.83 due to 2-H of the minor component (**2b**) showed clear correlation with carbons at the 1, 3, and 7 positions in the NMR spectra. The overall HMBC analyses depicted in Figure 1 led to the gross structure of the minor component (**2b**). The chemical shifts of eight different kinds of carbons and their multiplicities in the ^{13}C NMR spectra (Table 3) are consistent with the gross structure (**2b**). Each proton located at the geminal and vicinal positions was correlated properly by the aid of H-H COSY study in the ^1H NMR spectra of **2b**. The presence of clear correlation between 2- and 5-protons in the NOESY spectra of **2b** suggests the *cis* configuration of the succinimido moiety with respect to 5-methyl attached to the boat conformation.

^1H and ^{13}C NMR spectra of the major component (**2a**) are summarized in Table 2, and close resemblance of the two components in Tables 2 and 3 suggests that **2a** is a stereoisomer of **2b**. Although no clear evidence is available on the stereochemistry, *trans* relation of the succinimido moiety with respect to the methyl group is plausible, the two appendants being located equatorially on the δ-lactone with chair conformation. The absolute configuration of **2a** is deduced on the basis of that of L-lysine while that of **2b** is obscure at present since epimerization at the 2-position of **2a** during treatment with Ac_2O in warm pyridine is not completely ruled out, leading to the antipode of the stereostructure depicted for **2b**.

The compound (**3**), $C_9H_{12}O_6$ from HRMS, mp 87-88 °C (hexane/CH_2Cl_2), $[\alpha]_D^{27}$ -8.92° (c 0.5, $CHCl_3$), IR (CCl_4) 1804 and 1762 cm^{-1}. ^{13}C NMR ($CDCl_3$, 125 MHz) at δ 173.0 (*s*, C1), 74.9 (*s*, C2), 71.8 (*d*, C3), 69.9 (*t*, C4), 21.6 (*q*, C5), 169.4 and 169.6 (each *s*, CO), and 20.2 and 20.3 (each *q*, CH_3). ^1H NMR spectrum ($CDCl_3$, 500 MHz) is consistent with the assigned structure (**3**), in which 3-H at δ 5.35 (*dd*, 3.6

and 6.6 Hz) shows the clear NOEs with 2-CH_3 at δ 1.69 (*s*) and two protons of 4 position at δ 4.33 (*dd*, 3.6, 10.6) and 4.58 (*dd*, 6.6, 10.6).

The compounds (**1**, **2** and **3**), all of which were isolated in less than 10^{-4} % yields from the original plant, may be derived from the corresponding free acids (**1A**),[5] (**2A**) and (**3A**),[6] contained in the crude extracts, by the employed acetylation reaction.

The activity experiments are lacking at present, and synthesis of the presumed compounds (**1A**, **2A** and **3A**) followed by assay experiments are essentially needed, and these are in progress.[7]

Table 1 ^{1}H and ^{13}C NMR spectra of compound (**1**)

^{13}C NMR (125 MHz)		^{1}H NMR (500 MHz)		H-H COSY
C		**H**		
1	170.0 (*s*)			
2	47.3(*d*)	**2**	4.73 *ddd* (6.4, 8.6, 11.3)	9, 3a, 3b
3	24.4 (*t*)	**3a**	1.52 *dtd* (4.6, 11.3, 13.4)	2, 3b, 4a, 4b
		3b	2.64 *tdd* (6.1, 8.6, 13.4)	2, 3a, 4a, 4b
4	28.0 (*t*)	**4a**	1.72 *dtd* (6.1, 11.3, 14.3)	5, 3a, 3b, 4b
		4b	2.05 *dddd* (3.4, 4.6, 6.1, 14.3)	5, 3a, 3b, 4a
5	74.1 (*d*)	**5**	4.60 *dqd* (3.4, 6.1, 11.3)	4a, 4b, 6
6	20.8 (*q*)	**6**	1.40 *d* (6.1)	5
7	173.2 (*s*)			
8	23.0 (*q*)	**8**	2.40 *s*	
		NH	6.58 *br s*	2

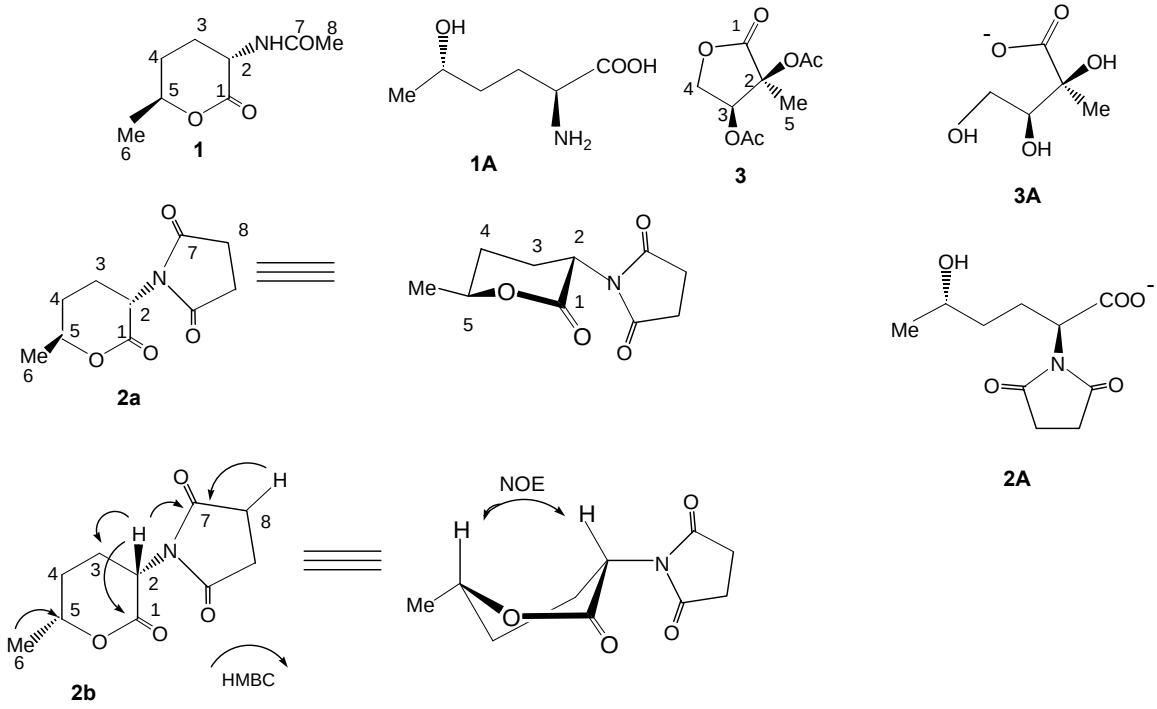

Figure 1. Structures of compounds (**1**, **2**, and **3**)

Table 2. NMR spectra of major product (**2a**)

^{13}C NMR (150 MHz) (*multi.*)		^{1}H NMR (600 MHz) *multi.* (J/Hz)			H-H COSY
C		**H**			
1	166.9 (*s*)				
2	48.3 (*d*)	**2**	4.68	1H, *m*	3a, 3b
3	24.5 (*t*)	**3a**	2.01	1H, *m*	2, 3b, 4a
		3b	2.33	1H, *m*	2, 3a, 4a
4	30.3 (*t*)	**4a**	1.76	1H, *m*	3a, 3b, 4b, 5
		4b	2.05	1H, *m*	3a, 3b, 4a, 5
5	78.6 (*d*)	**5**	4.68	1H, *m*	4a, 4b, 6
6	21.7 (*q*)	**6**	1.43	3H, *d* (6.4)	5
7	176.0 (*s*)				
8	28.1 (*t*)	**8**	2.78	4H, *br s*	

Table 3. NMR spectra of minor product (**2b**)

^{13}C NMR (150 MHz) (*multi.*)		^{1}H NMR (600 MHz) *multi.* (J/Hz)			H-H COSY
C		**H**			
1	167.6 (*s*)				
2	47.2 (*d*)	**2**	4.83	1H, *dd* (9.0, 9.6)	3a, 3b
3	21.0 (*t*)	**3a**	2.45	1H, *m*	2, 3b, 4a
		3b	2.07	1H, *m*	2, 3a, 4a
4	28.1 (*t*)	**4a**	1.91	1H, *m*	3a, 3b, 4b, 5
		4b	2.78	1H, *br s*	3a, 3b, 4a, 5
5	76.0 (*d*)	**5**	4.68	1H, *m*	4a, 4b, 6
6	20.8 (*q*)	**6**	1.48	3H, *d* (6.1)	5
7	175.9 (*s*)				
8	28.1 (*t*)	**8**	2.78	4H, *br s*	

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7. The present assay experiments were carried out at 25^{o}C for 4 days in a dark incubator with freshly hatched nematode of *Meloidogyne incognita* species under the kind guidance of Dr. Iwao Yuhara by courtesy of NIC Research Institute at Shisui, Chiba (Japan).