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ISOLATION AND STRUCTURE OF ACROMELIC ACID A AND B. NEW KAINOIDS OF CLITOCYBE ACROMELALGA.

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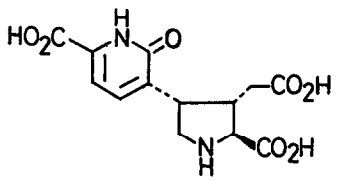
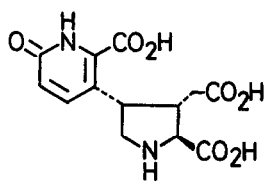
Abstract — A minute amount of the new kainic acid-like amino acids, acromelic acid A (ca. 110 μg) and B (ca. 40 μg), was isolated from the poisonous mushroom Clitocybe acromelalga and their structures were inferred to be 1 and 2 respectively by spectral analyses.

The poisonous mushroom Clitocybe acromelalga Ichimura is found in Japan only and exhibits unique symptoms similar to erythromelalgia or acromelalgia. Its remarkable physiological properties prompted us to study chemical constituents of this fungus.[1] Fractionation guided by the lethal effect on mice led to the isolation of a very minute amount of the amino acids structurally related to kainic acid and domoic acid.[2] We describe in this communication the isolation and characterization of these compounds, named acromelic acid A (1) and B (2).



acromelic acid A 1



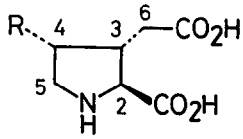
acromelic acid B 2

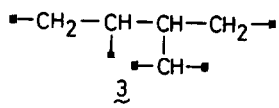
Water extract of 16.2 kg of fresh fruiting bodies was subjected to acetone precipitation, dialysis of the precipitate and the dialysate was then chromatographed successively on charcoal (2.5-5% EtOH), Amberlite IR-45 (HCO_2^- form, 10-20% HCO_2H), paper electrophoresis (pH 4.6, +90 mm) to give 52 mg of crude toxic fraction. Crude acromelic acids A and B were separated from the poisonous fraction by cellulose TLC ($nBuOH/HCO_2H/H_2O$=6/1/2, Rf 0.41 and 0.28 respectively), and purified by cellulose TLC ($nBuOH/AcOH/H_2O$=4/1/5) and finally by paper electrophoresis (pH 4.6). Amounts of purified acids A and B were about 110 μg and 40 μg respectively. Both compounds showed yellow coloration with ninhydrin, strong blue fluorescence under UV light and behavior of a strong acid comparable to cysteic acid on chromatography.

Due to the limited sample quantity, the spectral data available were ^1H NMR and UV spectra only.[3] The formulas 1 and 2, however, could be inferred from these data comparing them with those of related compounds.

The ^1H NMR spectra of both 1 and 2 (360 MHz, D_2O, DSS standard) were consisted of an AB pattern due to aromatic protons and signals ascribable to three methine and two methylene groups.

Table 1 J value, multiplicity (δ value)



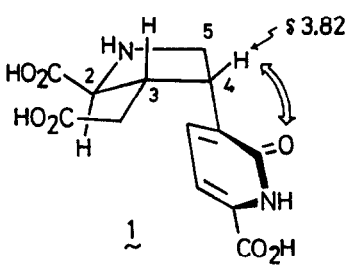


	H_2	H_3	H_4	H_5	$H_{5'}$	H_6	$H_{6'}$
1	4.13,d	3.12,dddd	3.82,q	3.73,dd	3.76,dd	2.01,dd	2.54,dd
	(8.1)	(5.4 7.1 8.1 10.8)	(7.1)	(7.1 12.0)	(7.1 12.0)	(10.8 16.5)	(5.4 16.5)
domoic acid	3.98,d	3.05,dddd	3.83,q	3.49,dd	3.70,dd	2.50,dd	2.75,dd
	(8.2)	(5.8 7.6 8.2 9.1)	(7.6)	(7.6 12.3)	(7.6 12.3)	(9.1 16.8)	(5.8 16.8)
2	4.16,d	3.15,ddt	4.47,dt	3.67,t	3.78,dd	2.23,d	
	(3.6)	(3.6 7.2 7.7)	(7.2 11.7)	(11.7)	(7.2 11.7)	(7.7)	
kainic acid	4.12,d	3.10,dddd	3.03,q	3.45,t	3.65,dd	2.40,dd	2.49,dd
	(3.6)	(3.6 6.3 7.2 8.3)	(7.2 11.7)	(11.7)	(7.2 11.7)	(8.3 16.6)	(6.3 16.6)

The sequence of the methine and methylene protons was verified to be 3 in both compounds by decoupling experiments. The presence of a proline moiety was indicated by the δ values for a methine (at δ 4.13 in 1 and 4.16 in 2) attributable to the C-2 proton, those for a methylene group (at δ 3.73, 3.76 in 1 and 3.67, 3.78 in 2) adjacent to nitrogen, and the color reaction with ninhydrin. The δ and J values of signals due to another methylene group (1: δ 2.01, 2.54, J=16.8 Hz; 2: δ 2.23) adjacent to carbonyl group, and the acidic character suggested the presence of a glutamic acid moiety. Taking all these facts into consideration, a structure of 4-substituted-2-carboxy-3-carboxymethyl pyrrolidine was inferred for each of 1 and 2. Indeed, as shown in Table 1, the ^{1}H NMR spectral data (δ and J) of 1 and 2 resembled closely those of domoic acid and and kainic acid respectively[4], except for the peaks due to the substituent at C-4. Almost same J values of the corresponding pyrrolidine ring protons suggested the same 2,3-trans-3,4-cis stereochemical structures for 1 and 2[5], a similar conformation for the pair of 1 and domoic acid and another similar conformation for the pair of 2 and kainic acid.

The UV spectra of both 1 and 2 (Table 2) appeared to be characteristic of 2-pyridone, in particular, of 2-pyridone-6-carboxylic acids.[6] Moreover, J values of 9 and 7 Hz are known to be indicative of J_{3-4} and J_{4-5} respectively in 2-pyridone-6-carboxylic acid derivatives.[7] Therefore, 3 and 5-substituted-2-pyridone-6-carboxylic acids were suggested as the structure of aromatic portion of 1 and 2, respectively. The above argument was supported by spectroscopic comparison with model compounds such as I, II and III[8], as shown in Table 2.

Fig. 1



That the pyrrolidine and pyridone portions thus far deduced were linked directly at C-4 position of the former to 3 or 5-position of the latter in 1 and 2 respectively was concluded by ^{1}H NMR analyses of the two aromatic doublet signals. In the both compounds the doublet due to H_4 appeared in lower height (~3/4) and broader width than another, (H_5 or H_3), indicating a small long-range coupling between H_4 of the pyrrolidine and H_4 of the pyridones.[9] Further support was obtained by studying conformation of 1 and 2. As main conformations 1 and 2 shown in Fig. 1 were deduced from J values and inspection of models. Anomalous down-field shift[11] of pyrrolidine H_4 peak of 1 (δ 3.82) and 2 (δ 4.47) was well rationalized from these models, since in 1 and 2, the carbonyl and carboxyl groups can approach close to H_4 respectively, as depicted in Fig. 1, to give the bond anisotropy effect. This interpretation implies direct linkage of the pyrrolidine and pyridone rings.

Thus acromelic acids A and B are best expressed by 1 and 2 respectively. These formulas are biogenetically also plausible, as shown in Fig. 2.[12]

Synthesis, absolute configuration and biological activities of these kainoids are now under investigation.

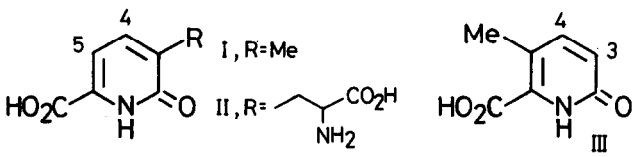
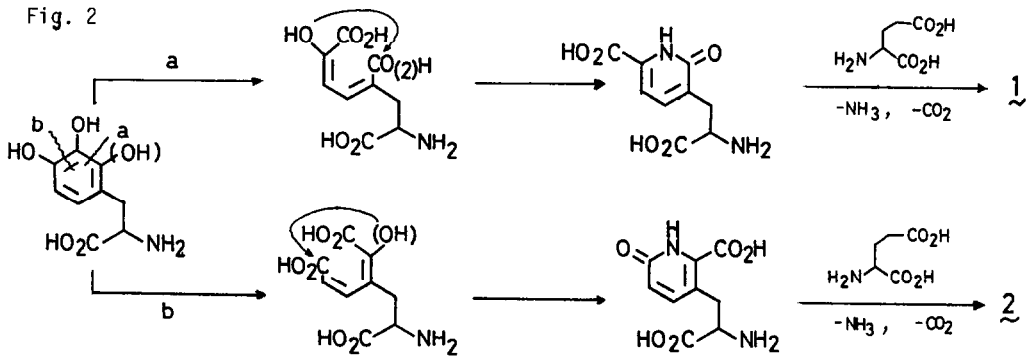


Table 2

	^{1}H NMR			λ_{max}		
	δ_4	$\delta_{5(3)}$	$J_{4\text{-}5(3)}$	pH 2	pH 7	pH 12
acromelic acid A	7.54	6.98	7.2	240, 313	242, 317	241, 312
I	7.62	6.96	7.0	241, 306	242, 310	245, 307
II	7.66	7.08	7.0	239, 311	241, 315	244, 311
acromelic acid B	7.63	6.68	9.3	231, 308	227, 300	241, 311
III	7.69	6.68	9.0	239, 310	235, 302	242, 313

Fig. 2



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References and Notes

1) a) K. Konno, K. Hayano, H. Shirahama, H. Saito and T. Matsumoto, Tetrahedron Letters, 1977, 481. b) K. Konno, H. Shirahama and T. Matsumoto, Tetrahedron Letters, 1981, 22, 1617.

2) Review: T. Takemoto, "Kainic Acid as a Tool in Neurobiology"; Raven Press: New York, 1978, p. 1; Physiological activity on the central nervous system: H. Shinozaki, ibid, p. 17.

3) ^{13}C NMR signals could not be observed even after 35912 transients. All attemps to measure the mass spectrum (FD-MS, SIMS) were unsuccessful.

4) The ^{1}H NMR spectral data of kainic acid and domoic acid (360 MHz, D_2O, DSS standard) were kindly informed from Dr. Y. Ohfune (Suntory Institute for Bioorganic Research). For synthesis of domoic acid: Y. Ohfune and M. Tomita, J. Am. Chem. Soc., 1982, 104, 3511.

5) The 2,3-trans structure was supported by the δ values for H_2 of 1 and 2. In the kainoids so far examined, when the 2,3-substituents (carboxyl and carboxymethyl) were trans oriented H_2 proton resonates at higher field than δ 4.2. On the other hand, in the cis-compounds it exhibits peaks at lower field than δ 4.2, irrespective of the substituent at C-4. For example, α-allo-kainic acid (KA), α-KA norketone and α-allo-KA norketone exhibited peaks at δ 3.94, 3.99 and 3.95, respectively, whereas β-KA, β-KA norketone and β-allo-KA norketone showed their respective lines at δ 4.34, 4.36 and 4.22 (400 MHz, D_2O, DSS standard). All these KA derivatives were prepared from α-KA by the known method: R. Nakamori, J. Pharm. Soc. Japan, 1956, 76, 265.

6) L. Rateb, G. A. Mina and G. Soliman, J. Chem. Soc. (c), 1968, 2140.

7) H. Weber, Arch. Pharm., 1976, 309, 664.

8) All model compounds were prepared from the corresponding pyrone derivatives by treatment with NH_3 aq.

9) In the spectra of the model compounds illustrated in Table 2, a clear long-range coupling (J ~1 Hz) was observed between H_4 and allylic protons on the side chain.

10) Conformations similar to 1 and 2 have been found for domoic acid and kainic acid respectively by NOE experiments: personal communication from Dr. Y. Ohfune,

11) Usually δ value of such proton is observed at δ 2.8-3.2 as in kainic acid.

12) a) H. Musso, Tetrahedron, 1979, 35, 2843, and references cited therein. b) Y. Saeki, M. Nozaki and S. Senoh, J. Biol. Chem., 1980, 255, 8465.

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