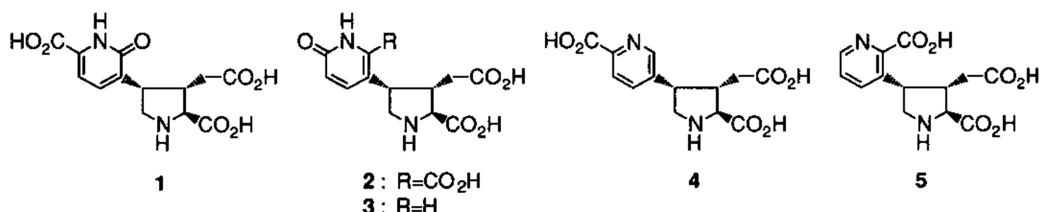
ISOLATION OF ACROMELIC ACIDS D AND E FROM *CLITOCYBE ACROMELALGA*

Shinji Fushiya, Seiichi Sato, Yasuko Kera, and Shigeo Nozoe*
Pharmaceutical Institute, Tohoku University, Aobayama, Aoba-ku, Sendai 980, Japan

Abstract --- Two analogs of acromelic acid were isolated from *Clitocybe acromelalga*. The structures of these compounds, acromelic acids D and E were determined to be **4** and **5**, respectively by ^{1}H nmr analysis and by comparison of the spectral data with that of the synthetic one in the case of **4**.

Acromelic acids A (AA-A) (**1**) and B (AA-B) (**2**)[1] are the toxic constituents isolated from *Clitocybe acromelalga*. They exhibit powerful neuroexcitatory activities[2] and are thought to be the active principles on the unique symptoms similar to acromelalgia and erythromelalgia responsible after ingestion of this toxic mushroom. The complexity of the symptoms which implies the presence of other active compounds in the mushroom prompted us to study on the toxic constituents, and we reported the isolation of acromelic acid C (AA-C) (**3**) in the previous paper.[3] Further investigation has revealed the presence of two minor acromelic acid analogs. Herein, we report the isolation and the structure of these compounds, acromelic acids D (AA-D) and E (AA-E).



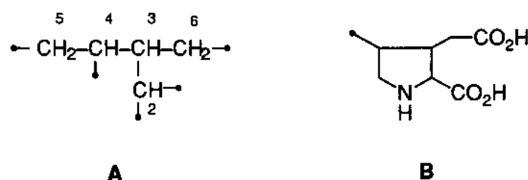
The acidic amino acid fraction I prepared from the aq. EtOH extract of *C. acromelalga* (8.1 kg)[3] was subjected to a Dowex 50W X4 column which was equilibrated with a pH 3.20 ammonia-

formate buffer, and the column was eluted with the same buffer. The fractions showing peaks at 18.0–20.0 min on hplc[4] were collected. Successive purification by hplc and cellulose tlc (nBuOH/AcOH/H_2O=4/1/2) gave AA-D (*Rf* 0.13, 2.5 mg) and AA-E (*Rf* 0.12, 0.3 mg).

AA-D, $[\alpha]_D$ +17.6° (*c* 0.07, H_2O), cd $[\theta]_{211}$ +2281, showed a yellow coloration with ninhydrin. The FAB-ms exhibited an ion peak at m/z 295 $(M+H)^+$, corresponding to the molecular formula $C_{13}H_{14}N_2O_6$. AA-E showed a yellow coloration with ninhydrin, too.[5] The ^1H nmr spectra of AA-D and AA-E exhibited signals due to two methylene and three methine groups, respectively (Table 1).

Table 1. ^1H nmr spectral data of AA-D and AA-E

	H2	H3	H4	H5a	H5b	H6a	H6b
AA-D	4.12, d (5.9)	3.28, dddd (5.9, 6.4, 8.3, 9.3)	4.06, ddd (7.8, 8.3, 10.4)	3.80, dd (10.4, 11.7)	4.01, dd (7.8, 11.7)	2.10, dd (9.3, 16.6)	2.46, dd (6.4, 16.6)
AA-E	4.23, d (2.4)	3.12, ddt (2.4, 5.9, 8.8)	3.94, dt (11.7, 8.8)	3.75, t (11.7)	3.83, dd (8.8, 11.7)	2.08, dd (8.8, 15.6)	2.13, dd (5.9, 15.6)

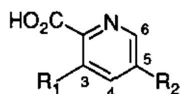


Analysis of these signals with the aid of decoupling experiments demonstrated that both AA-D and AA-E have the partial structure A. The chemical shifts and splitting patterns of these signals resemble closely to those of the pyrrolidine moieties of AA-A (**1**), AA-B (**2**)[1] and AA-C(**3**), indicating both compounds are kainoids. The partial structure A is thus extended to B.

Further, the uv absorption maxima (AA-D; 221.4 and 269.2 nm, AA-E; 266.8 nm) and the aromatic proton signals in the ^1H nmr spectra (Table 2) indicate that AA-D is 2,5-substituted and AA-E is 2,3-substituted pyridine,[6] which are deduced to be 5-substituted and 3-substituted picolinic acid, respectively from the molecular formula, the acidic properties and biogenetic consideration. The combination of two partial structures, respectively gave the structures **4** for AA-D and **5** for AA-E.

Table 2. ^{1}H nmr data of aromatic moieties of AA-D and AA-E

	H3	H4	H5	H6
AA-D	8.17, d (8.3)	8.23, brd (8.3)	–	8.57, brs
AA-E	–	7.42, dd (4.4, 7.8)	7.78, d (7.8)	8.38, d (4.4)

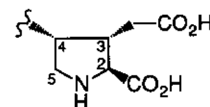


AA-D: R_1=H, R_2=pyrrolidine
AA-E: R_1=pyrrolidine, R_2=H

The 2,3-*trans* and 3,4-*cis* relationship of the pyrrolidine rings of AA-D and AA-E were deduced from the resemblance of the *J* values between AA-D and AA-B (**2**), and between AA-E and kainic acid, respectively (Table 3). Further, coexistence of AA-D and AA-E with AA-A (**1**), AA-B (**2**) and AA-C (**3**) in *C. acromelalga* suggests that both amino acids have 2(*S*) configuration.

Table 3. Comparison of *J* values of AA-D and AA-E with those of AA-B and kainic acid (KA)

	$J_{2\text{-}3}$	$J_{3\text{-}4}$	$J_{4\text{-}5a}$	$J_{4\text{-}5b}$	$J_{5a\text{-}5b}$
AA-D	5.9	8.3	10.4	7.8	11.7
AA-B	6.0	8.1	11.5	8.1	11.5
AA-E	2.4	8.8	11.7	8.8	11.7
KA	3.2	7.0	11.3	7.5	11.3



From the accumulated data it was concluded that AA-D and AA-E have the stereostructures **4** and **5**, respectively.

4 has already been synthesized by Shirahama *et al.* for the study on structure-activity relationship of acromelic acids.[7] The ^{1}H nmr spectrum and $[\alpha]_D$ of **4** were identical with those of the synthetic specimen ($[\alpha]_D$ +19.3°).

The pyridone moieties of AA-A (**1**) and AA-B (**2**) are suggested to be derived biosynthetically from *L*-DOPA *via* oxidative cleavage, recyclization, oxidation and conversion from pyrone to pyridone.[8]

It can therefore be assumed that AA-D (**4**) and AA-E (**5**) are the intermediates of this pathway. Further investigation on the isolation of the related compounds is under way.

ACKNOWLEDGEMENTS

We wish to thank Professor Haruhisa Shirahama (Hokkaido University) for the ^{1}H nmr spectrum of synthetic **4**. This work was supported by a Grant-in-Aid for General Scientific Research (No. 01470135) from the Ministry of Education, Science and Culture, Japan.

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4. Hplc was carried out on an instrument equipped with a refractive index detector and a uv detector. A stainless steel column (analytical: 0.26 x 50 cm, preparative: 1.0 x 50 cm) packed with Hitachi gel 2618 (cation exchange resin) was used and eluted with NH_3-HCO_2H buffer (analytical; pH 3.10, preparative; pH 3.50) at a rate of 0.5 and 3.0 ml/min, respectively.
5. The attempt to measure FD-ms was unsuccessful due to the limited sample quantity.
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Received, 26th March, 1992