Notes

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Hisashi Miyazaki, Yasuo Minaki, and Yoshio Yoshimura*1 : Preparation of Optically Active N-Carbamylaspartic Acid.

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The role of N-carbamyl-L-aspartic acid (L-C. A. A.) as a precursor of pyrimidines is well established.[1] Recently, Chiosa, *et al.*[2] and Cittadini, *et al.*[3] demonstrated the protective action of DL-C. A. A. against ammonia intoxication and the former authors discussed on the role of this compound in ammonia detoxication in relation to the control of the urea cycle and the pyrimidine biosynthesis. They expected a greater detoxication activity of the L-isomer, the naturally occurring form, than the DL-form with which they studied. However, in spite of the attempts[4,5] to obtain the optically active C. A. A., it

Table I. Properties of Optically Active N-Carbamyl-aspartic Acid

No.	N-Carbamyl-Aspartic acid	m.p. (uncorr.)	$[\alpha]_D^{20}$	C in H_2O	Hygroscopicity	Appearance of preparation
1	L-Free acid	128~130	+18.9	(3.38)	−	c. w. p.[a]
2					+	h. c.[b]
3	L-K_2-Salt		+16.6	(7.70)	+++	c. w. p.
4	L-Mg-Salt		+27.9	(2.95)	+	c. w. p.
5	L-Ca-Salt		+32.2	(2.53)	−	c. w. p.
6	L-Ba-Salt		+23.6[c]	(1.75)	−	c. w. p.
7	D-Free acid	128~130	−18.6	(0.640)	−	c. w. p.
8	D-Ca-Salt		−31.5	(1.54)	−	c. w. p.

No.	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
1	$C_5H_8N_2O_5$	34.09	4.58	15.91	33.99	4.60	15.88
2							
3	$C_5H_6O_5N_2K_2 \cdot H_2O$	22.20	2.98	10.37	22.12	2.97	9.84
4	$C_5H_6O_5N_2Mg \cdot H_2O$	27.74	3.72	12.94	27.58	3.93	12.75
5	$C_5H_6O_5N_2Ca \cdot H_2O$	25.86	3.48	12.07	25.89	3.14	11.76
6	$C_5H_6O_5N_2Ba \cdot H_2O$	18.22	2.45	8.50	18.00	2.60	8.38
7	$C_5H_8O_5N_2$	34.09	4.58	15.91	34.10	4.55	15.89
8	$C_5H_6O_5N_2Ca \cdot H_2O$	25.86	3.48	12.07	25.85	3.20	11.77

a) crystalline white powder. *b*) hemispheric crystals.
c) The specific rotation appeared in the literature is $[\alpha]_D^{25} = +24.1$[4]

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1) P. Reichard : "Advances in Enzymology" Vol. 21, p. 263 (1959). Interscience Publishers, Inc., New York.
2) L. Chiosa, V. Niculescu, C. Bonciocat, C. Stancu : Biochem. Phamacol., **14**, 1635 (1965).
3) D. Cittadini, D. DeChristofaro, C. Balestrieri, F. Cimino : *Ibid.*, **15**, 992 (1966).
4) P. Reichard, U. Lagerkvist : Acta Chem. Scand., **7**, 1207 (1953).
5) I. Lieberman, A. Kornberg : J. Biol. Chem., **207**, 911 (1954).

has not been isolated in a crystalline form except for the barium salt.[4] Therefore, the physical and chemical properties of the optically active C.A.A. have been unknown while the racemic compound is easily available by two procedures.[6,7]

This paper describes the preparation of L-C.A.A. from L-aspartic acid according to the modified cyanate procedure.[7] The repeated purifications using the cation exchange resin were performed since the purification by absorption on the anionic resin, as described in the literatures,[4,5] appeared to be unavoidable to afford the contamination by the materials used for the elution from the resin. L-C.A.A. was obtained as crystalline white powder or large hemispherical crystals. Similarly, D-C.A.A. could be obtained from D-aspartic acid. A few kinds of their corresponding salts were also prepared. Their physical and chemical properties are listed in Table I together with their elemental analytical data. Unlike the racemate, these optically active acids are freely soluble in water and alcohol. In the concentrated solution or in the presence of the strong acid, gradual cyclization occurs to form 5-carboxymethylhydantoin. From the combined solution of the D- and L-isomers were yielded crystals with m.p. 178~180°, which corresponded to the racemate.[7]

The biological study of the optically active C.A.A. will be reported elsewhere.

Experimental

Preparation of Free Acid——According to the method of Nyc and Mitchell,[7] 20 g. of L-aspartic acid was dissolved in 150 ml. of 1*N* potassium hydroxide and 12.3 g. of potassium cyanate was added to the solution. After 16 hr. 400 ml. (wet vol.) of cation exchange resin, Duolite C-25 (H-form), was added to the solution (solution 1) and the mixture was stirred for 30 min. Then the resin was filtered off and washed with 130 ml. of water. The filtrate and the washing were combined (solution 2), concentrated to about 50 ml. under reduced pressure at 40~45° and passed through the column of 40 ml. of Duolite C-25 (H-form) with the flow rate of 10 ml./min. The column was then washed with water. The initial 20 ml. of the effluent was discarded and 100 ml. of the following effluent (solution 3) was collected. After the addition of calcium carbonate (about 50 mg.), the solution was concentrated. The resultant syrup was rapidly dried over phosphorus pentaoxide *in vacuo* at room temperature and then dissolved in 20 ml. of EtOH. Small amount of calcium salt, contaminated with blue-colored matter yielded during the purification steps, was filtered off. To the clear filtrate (solution 4) was added 3 vol. of ether and the solution was chilled overnight, and 7.2 g. of L-C.A.A. was precipitated as crystalline white powder. Fig. 1 shows the paper chromatograms of the solution of each purification step.

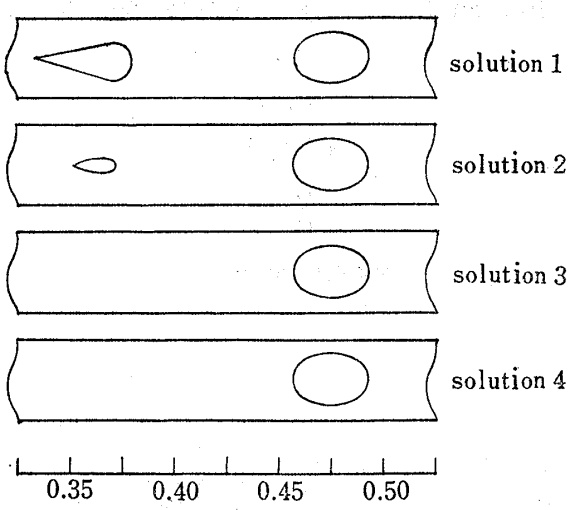

Fig. 1. Paper Chromatograms of the Solution 1~4 at each Purification Step

Solvent system, BuOH:AcOH:H_2O (2:1:1). Each spot represents Ehrlich's reagent[8] positive (N-carbamyl-aspartic acid, Rf 0.475) and ninhydrin positive (aspartic acid, Rf 0.370) spot, respectively.

When the powder thus obtained was recrystallized from acetone, hemispheric crystals just like buttons, with 0.5~1.5 cm. diameter were yielded. The D-isomer was prepared from D-aspartic acid in the quite same way.

Preparation of Salts——Seventy-five milliliter of an ethanolic solution of carbonate-free 1*N* potassium hydroxide was dropped into the ethanolic solution of L-C.A.A. (solution 4 in the purification steps of the free acid) and the mixture was shaken vigorously. The dipotassium salt was obtained as highly hygroscopic powder (Yield 9.65 g.).

The aqueous solution 3 was concentrated to about 50 ml., and 5 g. of magnesium hydroxide or 7.5 g. of calcium carbonate was added to the solution, which was then filtered. Small amount of precipitate, which

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adsorbed the blue matter, was formed by the addition of the small portion of EtOH. After filtration, EtOH was further added to the filtrate and 10.4 g. of magnesium salt or 11.4 g. of calcium salt was obtained. Barium salt of the L-isomer and calcium salt of the D-isomer were prepared in a similar way.

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Hans Bohrmann, Egon Stahl,*1 and Hiroshi Mitsuhashi*2 : Studies of the Constituents of Umbelliferae Plants. XIII.*3 Chromatographic Studies on the Constituents of *Cnidium officinale* MAKINO.

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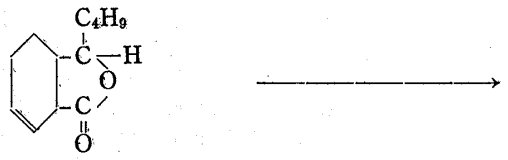
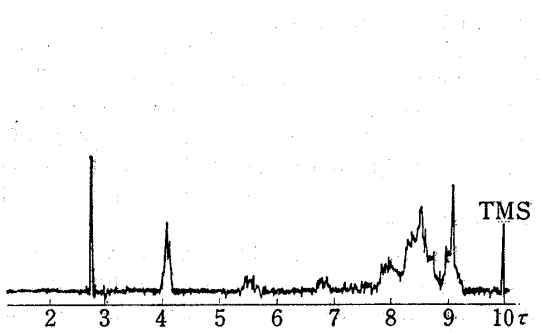
The structure determination of cnidilide and neocnidilide, phthalides from *Cnidium officinale* MAKINO, was reported in a previous paper of this series.[1] Since the repeated chromatography of the lactone mixture always gave cnidilide with a contamination of about 10% of butylphthalide, it was suggested, that cnidilide might aromatize spontaneously (Chart 1).

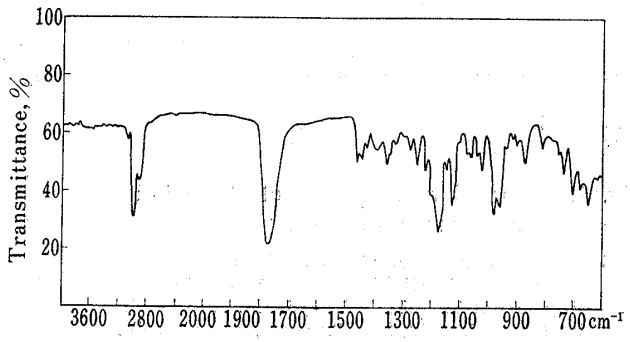


cnidilide butylphthalide

Chart 1.



Nuclear Magnetic Resonance Spectrum of Cnidilide (in $CDCl_3$)



Infrared Spectra of Cnidilide (neat)

Fig. 1.

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*3 Part XII : This Bulletin, **14**, 777 (1966).

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