Communications to the Editor

UDC 547.466.2

Reaction of Hydrazoic Acid with Dialkylmalonic Acids

Earlier report from this laboratory[1] showed that amino acids were obtained from monoalkylmalonic acids in a high yield by the Schmidt reaction. The present writer found that an abnormal reaction occurred in using dialkylmalonic acids as the starting material and a mixture of amino acids were obtained, instead of the expected amino acid.

Procedure of this reaction was as follows: A mixture of 1.6 g. of diethylmalonic acid, 5 cc. of 100% H_2SO_4, and 10 cc. of $CHCl_3$ was kept at 50~60°, 8 cc. of 6.5% hydrazoic acid in $CHCl_3$ was slowly added, and the mixture was maintained at the same temperature for several hours. The reaction mixture was then poured into ice water, the aqueons layer was separated, and carefully adjusted to pH 5 with hot concentrated solution of $Ba(OH)_2$. $BaSO_4$ that precipitated was removed and the filtrate was passed through Amberlite IR-120 column. The amino acid adsorbed was readily eluted from the column with 0.15~0.5*N* NH_4OH. Free amino acid was obtained by the concentration of the eluate. Yield, 0.15 g.

The product was easily purified by sublimation, but two spots (Rf 0.37, 0.63: solvent—BuOH: AcOH: H_2O=4:1:1) were observed on the paper chromatogram. Using paper chromatography, these amino acids were separated and respectively identified as DL-2-aminobutyric acid (Rf 0.37; Yield, 0.07 g. *Anal.* Calcd. for $C_4H_9O_2N$: C, 46.59; H, 8.80; N, 13.58. Found: C, 46.76; H, 8.96; N, 13.67) and 2-ethyl-2-aminobutyric acid (Rf 0.63; Yield, 0.05 g. *Anal.* Calcd. for $C_6H_{13}O_2N$: C, 54.93; H, 9.99; N, 10.68. Found: C, 55.15; H, 10.12; N, 10.90).

In the case of ethylisobutylmalonic acid, the reaction mixture showed three spots on the paper chromatogram.

Further study is being made to find out the mechanism of this abnormal reaction and detailed description on this work will be reported in the near future.

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1) Paper read before the General Meeting of the Pharmaceutical Society of Japan, April 8, 1956.

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Constituents of *Chondria armata*

A crystalline acid component was isolated from a seaweed, *Chondria armata* (KÜTZING) OKAMURA (Japanese name "Hanayanagi" or "Domoi") (family Rhodomelaceae), and was named domoic acid. It formed colorless needles of m. p. 217° (decomp.); $[\alpha]_D^{12}$ −109.6° (c=1.314, H_2O); U. V. $\lambda_{max}^{H_2O}$: 242 mμ (log ε 4.42) (*Anal.* Calcd. for $C_{15}H_{21}O_6N \cdot 2H_2O$: C, 51.86; H, 7.25; N, 4.03; 2C-CH_3, 8.66; 2H_2O, 10.37; mol. wt., 347.36. Found; C, 51.60, 51.71, 51.89; H, 7.11, 7.17, 7.16; N, 4.08, 4.08, 4.34; C-CH_3 (Kuhn-Roth method), 7.48, 6.97; H_2O, 10.13; mol. wt. (modified Akiya method), 366). Domoic acid is soluble in water and AcOH, easily soluble in dil. mineral acids and alkali hydroxide solutions, and soluble in alkali carbonate and $NaHCO_3$

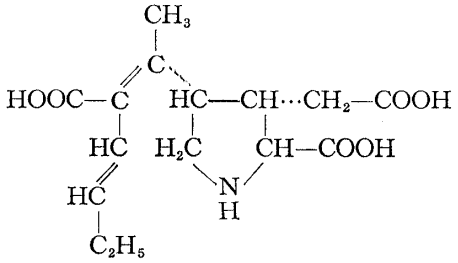
solution with foaming. The pKa's of its aqueous solution are 2.10, 3.72, 4.93, and 9.82. It is sparingly soluble in MeOH and EtOH, and insoluble in petr. ether, Et_2O, $CHCl_3$, benzene, and Me_2CO. The acid forms salts sparingly soluble in water with Cu, Pb, and Hg. Domoic acid rapidly discolors Br_2 water and $KMnO_4$ solution, showing unsaturation. Its infrared spectrum exhibits absorption at 10.30 μ for a *trans*-disubstituted C=C bond.

Catalytic reduction of domoic acid with Adams' PtO_2 catalyst resulted in absorption of 2 moles of H_2 to form tetrahydrodomoic acid, m.p. 273° (decomp.), $[\alpha]_D^{20}$ −11.8° (c=1.01, 1*N* HCl) (*Anal.* Calcd. for $C_{15}H_{25}O_6N$: C, 57.13; H, 7.99; N, 4.44; 2C-CH_3, 9.54. Found: C, 56.63; H; 7.92; N, 4.21; C-CH_3 (Kuhn-Roth method), 7.18). Its ultraviolet absorption lacked the band at 242 mμ and the infrared spectrum showed no absorption at 10.30 μ, present in the original acid.

Domoic acid colors yellow to ninhydrin ($\lambda_{max}^{H_2O}$ 403 mμ, $\lambda_{max}^{iso\text{-}AmOH}$ 413 mμ) and gives blue coloration to Feigl's aliphatic secondary amine reaction. Application of Ac_2O to aqueous solution of domoic acid afforded N-acetyldomoic acid, m. p. 121~123°; $[\alpha]_D^{20}$ −56.0° (c = 1.00, H_2O); $\lambda_{max}^{H_2O}$ 242 mμ (log ϵ 4.48) (*Anal.* Calcd. for $C_{17}H_{23}O_7N \cdot 2H_2O$: C, 52.43; H, 6.99; N, 3.60; $2H_2O$, 9.25. Found: C, 52.24; H, 6.97; N, 3.51; H_2O, 8.83). N-Acetyldomoic acid was negative to the ninhydrin reaction and consumed 3 equivalents of alkali for neutralization.

Cold ozonolysis of domoic acid in aqueous solution afforded L$_s$-*arabo*-2-carboxy-3-carboxymethyl-4-acetylpyrrolidine,[1] m.p. 197°, $[\alpha]_D^{20}$ +62.6° (c=1.09, H_2O), and propionaldehyde, identified as its 2,4-dinitrophenylhydrazone of m. p. 153°. Both were identified with authentic samples.

Based on foregoing observations, the structure of domoic acid was established as L$_s$-*arabo*-2-carboxy-3-carboxymethyl-4-(1-methyl-2-carboxy-1, 3-hexadienyl) pyrrolidine (I).



(I) Domoic acid

Oral administration of a single dose of 20 mg. of domoic acid showed a marked anthelmintic effect, equal to or better than that of kainic acid-santonin compound preparation (10 mg. kainic acid+50 mg. santonin).[2] It should especially be noted that domoic acid effected expulsion of a large number of pinworm (*Enterobium vermicularis* L.).

Examinations were made on the assay method for domoic acid and a few good methods were established, which included colorimetric determination of the color developed by imino group reagents, such as ninhydrin, alloxane, or 1,2-naphthoquinonesulfonic acid, and measurement of absorbance of a solution containing domoic acid at 242 mμ.

Free amino acid in *Chondria armata* was examined by paper partition chromatography and spots corresponding to aspartic acid, glutamic acid, serine, glycine, threonine, alanine, citrulline, histidine, valine, and tyrosine were detected. Pure samples were isolated of D-aspartic acid, m. p. 260° (decomp.), $[\alpha]_D^7$ −22.8° (c=2.90, 1*N* HCl), $[\alpha]_D^7$ +3.0° (c=2.67, 1*N*=NaOH), and L-citrulline, m.p. 222° (decomp.), $[\alpha]_D^{11}$ +4.1° (c=2.91, H_2O), $[\alpha]_D^{11}$ +15.3° (c=2.51, 2*M* HCl). The presence of D-aspartic acid was determined by comparison with Lutz's pH-specific

1) S. Murakami, T. Takemoto, Z. Tei, K. Daigo: Yakugaku Zasshi, **75**, 866 (1955); R. Nakamori: *Ibid.*, **76**, 275, 545 (1956).
2) T. Takemoto, Z. Shimizu, K. Daigo, T. Sai: Yakugaku Zasshi, **74**, 107 (1954); T. Takemoto, Z. Shimizu, K. Daigo, T. Sai: Yakugaku Kenkyu, **26**, 105 (1954).

rotation curve and this is an interesting example of a discovery of D-amino acid from vegetable world.

Detailed report of this work will be published in the near future.

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Über die Konfiguration am Kohlenstoffatom-22 des natürlichen 22-Hydroxycholesterins

Vor kurzem hat Hayatsu,[1] einer von uns, die Herstellung des 22-Hydroxycholesterins beschrieben, welches neben Carotinoidfarbstoffen aus einer Lilienart *Narthecium ossifraga* HUDS. gewonnen wurde.[2] Wir berichten hier über die Versuche zur Konfigurationsaufklärung des Kohlenstoffatoms 22 dieses Sterins.

Die durch $NaBH_4$-Reduktion aus 22-Oxocholesteryl-acetat (I) gewonnenen isomeren 22-Ole wurden nach Hayatsu sofort in 3,22-O-Dibenzoate verwandelt und der chromatographischen Trennung unterworfen; einer von zwei erhaltenen Dibenzoate erwies sich als identisch mit einem von Stabursvik isolierten Stoff. In vorliegender Arbeit haben wir zwei isomere 22-Ole, A und B, in reiner Form als Kristallisat erhalten: Isomere-A (IIa), Schmp. 185~186°, $[\alpha]_D$ −39.0° (Diacetat, Schmp. 102~103°, $[\alpha]_D$ −37.1°; Dibenzoat, Schmp. 254~256°, $[\alpha]_D$ −9.6°); Isomere-B (IIb), Schmp. 180~182°, $[\alpha]_D$ −52.0° (Diacetat, Schmp. 145~146°, $[\alpha]_D$ −51.5°; Dibenzoat, Schmp. 169~172°, $[\alpha]_D$ −19.6°). Dieses Isomere-A erwies sich nach Schmp., Mischprobe, Drehung und I.R.Spektrum als identisch mit natürlichen Stoff.

Zur Konfigurationsbestimmung des asymmetrischen Kohlenstoffs 22 von A bzw. B eignen sich die asymmetrische Synthese nach Prelog,[3] da sich die drei Reste an C-22 des 22-Ols durch ihre Grösse stark unterscheiden. Wir haben deshalb 3β-O-Methyläther, (IIIa) und (IIIb), hergestellt und sie in ihren Phenylglyoxylsäureester übergeführt. Diese letzteren wurden unter gleichen Bedingungen mit Methylmagnesiumjodid umsetzt und die entstandenen Atrolactinsäure-ester alkalisch vollständig verseift. Hierbei gab das (IIIa) eine rechtsdrehende Atrolactinsäure (vgl. Tabelle I).

TABELLE I.

Alkohol	Atrolactinsäure[a]		
	Ausbeute (%)	$[\alpha]_D$ [b]	p (%)[c]
(IIIa)	70	+ 7.0°	18
(VIa)	45	+ 2.9°	7.7
(IIIb)	52	−12.6°	33.4
(VIb)	48	− 3.8°	10
4,4-Dimethylcholesterin	62	+ 9.4°	25

1) R. Hayatsu: Dieses Bulletin, **5**, 452(1957).
2) A. Stabursvik: Acta Chem. Scand., **7**, 1220(1953).
3) V. Prelog: Helv. Chim. Acta, **36**, 308(1953).