Notes

[Chem. Pharm. Bull. 28(10)3114—3117(1980)]

Identity of the Synthetic Carbocylic Analog of Adenosine and Aristeromycin

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(Received February 18, 1980)

Comparisons of aristeromycin and the synthetic carbocyclic analog (C-Ado) of adenosine by several methods have been made because of anomalous results relating to aristeromycin that have appeared in the literature. Aristeromycin and C-Ado demonstrated the same behavior in four thin-layer chromatography and three high-pressure liquid chromatography solvent systems. Proton (100 MHz) and carbon-13 (25 MHz) nuclear magnetic resonance spectra of the two substances, determined under the same experimental conditions, are identical. Previously, comparisons of certain data had supported the identity of aristeromycin and C-Ado; the data presented here show unequivocally that the synthetic and natural forms of the carbocyclic analog of adenosine are identical.

Keywords——aristeromycin HPLC and TLC; adenosine carbocyclic analog HPLC and TLC; C-Ado; aristeromycin NMR; adenosine carbocyclic analog NMR; aristeromycin and adenosine carbocyclic analog identity

In 1966, a multistep synthesis of the racemic carbocyclic analog[2] (C-Ado, Ia+Ib of Chart 1) of adenosine was first reported by Shealy and Clayton.[3a] Soon thereafter, aristeromycin was isolated,[4] and the structure of this antibiotic was reported by Kishi *et al.*[5] to be the 1′*R*, 2′*S*, 3′*R*, 4′*R*-isomer[6] (Ia) of C-Ado. At that time, our groups exchanged infrared and 60-MHz proton nuclear magnetic resonance (NMR) spectra of C-Ado and aristeromycin and concluded that the two materials were identical.[5b] Subsequently, a different synthesis of C-Ado was reported,[7] and resolution into the (+) and (−) forms by chromatography on cellulose was claimed. Apparently, however, there were differences between the circular dichroism spectra of aristeromycin and the resolved forms.[7] In addition, the melting points of the resolved isomers (120—121° and 142—144°) differ markedly from that of aristeromycin (213—215° dec., ref. 5*b*); the mp (>260°) of "2′,3′-*O*-isopropylidene-(±)-aristeromycin"[7] differs from the mp (216°) of isopropylidene-C-Ado;[3b] and the proton NMR data reported[3b] for C-Ado and isopropylidene-C-Ado differ significantly from the proton NMR data reported[7] for "(±)-aristeromycin" and "2′,3′-*O*-isopropylidene-(±)-aristeromycin" obtained by the new synthesis. Because of these apparent discrepancies, we have made a comparison in depth of the properties of C-Ado and aristeromycin.

Both thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC) indicated that C-Ado and aristeromycin are identical. The two compounds traveled identical-

1) Location: *2000 Ninth Avenue, South, Birmingham, Alabama, 35255, U.S.A.*
2) (±)-(1α,2α,3β,5β)-3-(6-Amino-9*H*-purin-9-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (*Chemical Abstracts* numbering. See non-primed numbers of structure Ia.).
3) *a*) Y.F. Shealy and J.D. Clayton, *J. Am. Chem. Soc.*, **88**, 3885 (1966); *b*) *Idem, ibid.*, **91**, 3075 (1969).
4) T. Kusaka, H. Yamamoto, M. Shibata, M. Muroi, T. Kishi, and K. Mizuno, *J. Antibiot.* (Tokyo), *Ser. A*, **21**, 255 (1968).
5) *a*) T. Kishi, M. Muroi, T. Kusaka, M. Nishikawa, K. Kamiya, and K. Mizuno, *Chem. Commun.*, **1967**, 852; *b*) *Idem, Chem. Pharm. Bull.*, **20**, 940 (1972).
6) Nucleoside numbering system. See primed numbers of structure Ia.
7) A. Holý, *Collect. Czech. Chem. Commun.*, **41**, 2096 (1976).

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Table I. Thin-Layer Chromatography of Aristeromycin and C-Ado[a]

Developing solvent	Detection method	Aristeromycin		C-Ado	
		Amt. Applied[b]	Rf	Amt. Applied[b]	Rf
Chloroform–methanol, 3:1	UV,[c] Charring with $(NH_4)_2SO_4$	10	0.28	12	0.28
Butanol–water, 86:14	UV, basic $KMnO_4$spray	10	0.37	12	0.38
		20	0.38	24	0.38
Butanol–water-acetic acid, 5:3:2	UV, basic $KMnO_4$ spray	10	0.64	12	0.65
		20	0.64	24	0.64
Ethanol–15N NH_3, 4:1	UV, basic $KMnO_4$ spray	10	0.69	12	0.69
		20	0.70	24	0.70

a) Water solutions of aristeromycin and C-Ado applied side-by-side on the plates. *Rf*=distance from origin to approximate center of spot/distance from origin to front.
b) Micrograms.
c) Examined with a UV lamp emitting principally at 254 nm.

Table II. Comparison of Aristeromycin and C-Ado by HPLC[a]

Developing Solvent	Aristeromycin		C-Ado		Artificial mixture of aristeromycin and C-Ado		
	%	Retention time	%	Retention time	%	Retention time	
Water–acetonitrile, 92:8	98.6	8.0	99.2	7.95			
	1.3	6.3[b]	0.8	4.7[b]			
80% Tris–phosphate buffer+20% Methanol[c]	98.8	6.7	99.4	6.7		6.7	
	1.2	5.7[b]	0.6	4.7[b]		4.6,[b]	5.7[b]
0.1 M Ammonium acetate–acetonitrile, 98:2	98.4	5.95	100[d]	5.98	99.3[e]	5.77	
	1.6	5.08[b]			0.7	4.97[b]	

a) Detection by UV absorption at 254 nm. Flow rate, 1 ml/min.
b) Impurity.
c) Solution composed of 20% methanol and 80% 0.1 M tris(hydroxymethyl)aminomethane to which H_3PO_4 was added to pH 6.8.
d) This specimen of C-Ado was purer than that used for the first two determinations.
e) 50:50 mixture.

ly during TLC in four different solvent systems (Table I). HPLC (Table II) showed that both specimens, as well as an artificial mixture of the two, had identical retention times. (The specimen of aristeromycin and one of the specimens of C-Ado contained small amounts (0.5—1%) of different impurities.) The mass spectra of the two compounds showed the same peaks, and the relative intensities were the same within normal experimental variation. The proton NMR and carbon-13 NMR, determined under the same experimental conditions, confirm unequivocally the identity of the two specimens. The carbon-13 NMR data are listed in Table III. The proton NMR spectra of aristeromycin and C-Ado, reproduced in Fig. 1 and 2, are identical. Thus, there is no doubt concerning the identity of aristeromycin and C-Ado.

C-Ado was synthesized[3] by a stereospecific route from *exo*-bicyclo[2.2.1]hept-5-ene-2,3-diol (II), a compound with fixed geometry. The structure of this starting material was established unequivocally by chemical and NMR methods.[3b,8] From II, (±)-(1α, 3α, 4β, 5β)-4,5-

8) M.C. Thorpe and W.C. Coburn Jr., *J. Org. Chem.*, **34**, 2576 (1969).

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Table III. Carbon-13 NMR Spectra of C–Ado and Aristeromycin[a]

C–Ado	Aristeromycin	Position[b]
29.28	29.26	C4
45.39	45.39	C5
59.41	59.41	C3
62.98	62.98	CH_2OH
71.71	71.71	C1[c]
74.62	74.62	C2[c]
119.35	119.35	P5
140.00	140.00	P8
149.75	149.75	P4
152.01	152.03	P2
155.96	155.96	P6

a) In DMSO-d_6 at concentrations shown in Fig. 1; tetramethylsilane as internal reference.
b) C1—C5 are carbon atoms in the cyclopentane ring shown with non-primed numbers in structure Ia. P2—P8 are carbon atoms in the purine ring and are designated by the conventional purine numbering system.
c) These assignments may be interchanged.

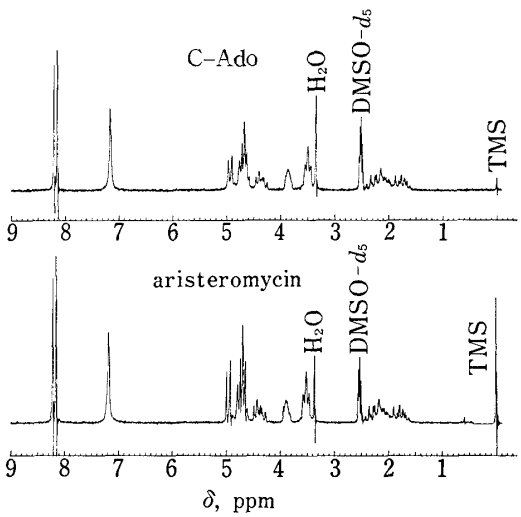

Fig. 1. Proton NMR Spectra of C–Ado (19 mg/0.4 ml) and Aristeromycin (26 mg/0.4 ml) in DMSO-d_6; 100 MHz, Tetramethylsilane as Internal Reference

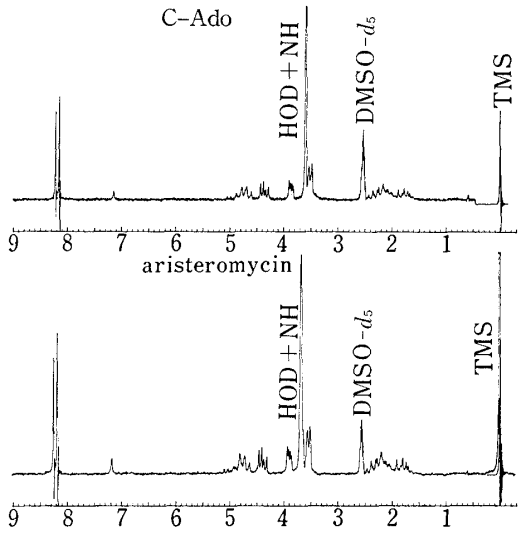

Fig. 2. Proton NMR Spectra of C–Ado and Aristeromycin in DMSO-d_6+D_2O (Concentrations as in Fig. 1+D_2O); 100 MHz, Tetramethylsilane as Internal Reference

(diacetyloxy)-1,3-cyclopentanedicarboxylic acid (III), a cyclopentane having the required geometric arrangement of functional groups, was obtained and was converted to the essential aminocyclopentane (IV). At the end of the stereospecific route from *exo*-bicyclo[2.2.1]hept-5-ene-2,3-diol (II), the structure of C-Ado was further confirmed by the formation of isopropylidene and cyclonucleoside-type derivatives of Iab.[3] The structure of aristeromycin was assigned[5] on the basis of physical data and studies of its pentaacetate. An X-ray analysis of the hydrobromide indicated that the absolute configuration is 1′*R*, 2′*S*, 3′*R*, 4′*R*.[5] Thus, these earlier studies by one of the groups had firmly established the structure of C-Ado as the racemic form of the carbocyclic analog of adenosine, and studies by the other group had strongly indicated that aristeromycin is an enantiomer. The data summarized here unequivocally confirm the identity of the synthetic and the natural forms.

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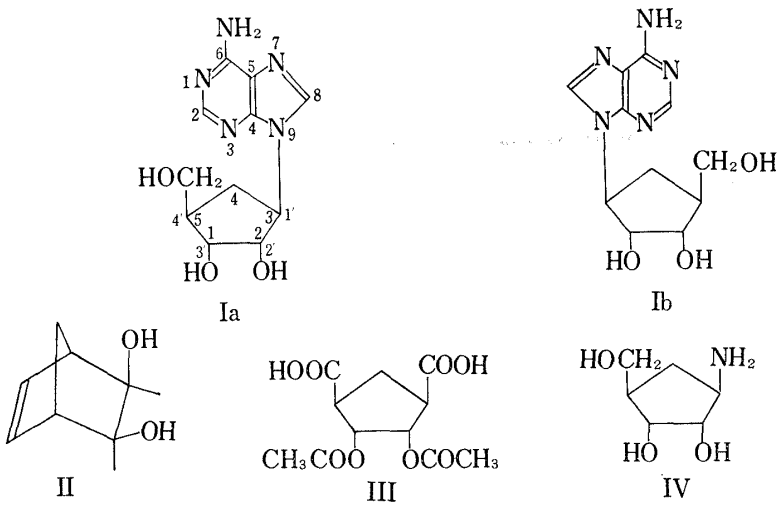

Chart 1

Experimental[9]

Thin-layer chromatography was performed on plates of silica gel G, 0.25 mm in thickness (purchased from Analtech, Inc., Blue Hen Industrial Park, Newark, New Jersey, U.S.A.). High-pressure liquid chromatography was performed on μBondapak C18 columns (Waters Associates, Milford, Massachusetts, U.S.A.). Mass spectra were determined with a Varian MAT Model 311A spectrometer at 70 eV, direct-probe temperature 20°; M=molecular ion. Proton NMR spectra at 100 MHz and carbon-13 NMR spectra at 25 MHz were determined with a Varian model XL-100-15 spectrometer equipped with a Gyrocode spin decoupler and a Digilab model NMR-3 data system and pulser for the determination of pulsed, Fourier-transform spectra.

Proton NMR——Chemical shifts quoted for multiplets are measured from the approximate centers; ranges are given for overlapping multiplets. Proton NMR of both C-Ado and aristeromycin in DMSO-d_6 (*cf.* Fig. 1, tetramethylsilane as internal reference, cyclopentane-ring protons identified by non-primed numbers of structure Ia): 1.5—2.5 (multiplet, H4α, H4β, H5), 3.51 (multiplet, CH_2 of CH_2OH), 3.88 (multiplet, H1), 4.36 (multiplet, H2), 4.5—4.9 (multiplet, H3, OH at C1, and OH of CH_2OH), 4.95 (doublet, OH at C2), 7.18 (NH_2), 8.15 and 8.22 (singlets, H2 and H8 of purine ring).

Mass Spectra (Fragment, Relative Intensity of C-Ado, Relative Intensity of Aristeromycin)——*m/e* 265 (M, 4, 5), 248 (M–OH, 3, 2), 247 (M–H_2O, 3, 2), 234 (M–CH_2OH, 6, 5), 218 (4, 2), 216 (4, 2), 206 (6, 3), 190 (8, 6), 178 (7, 4), 162 (32, 29), 148 (10, 6), 136 (100, 100), 135 (58, 50), 119 (11, 7), 108 (30, 21), 81 (17, 10).

Acknowledgement We thank Mr. Marion C. Kirk, Southern Research Institute, for mass spectral determinations and Mrs. Anita T. Shortnacy, Southern Research Institute, for HPLC determinations. The authors are very grateful to Dr. Toyokazu Kishi, Research and Development Division, Takeda Chemical Ind., Ltd., Osaka, Japan, for the gift of a specimen of aristeromycin.

9) The specimen of aristeromycin used in this study was kindly provided by Dr. Toyokazu Kishi.

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