[Chem. Pharm. Bull. 16(7)1383—1387(1968)]

UDC 547.455.083 : 543.544.25

Gas Chromatography of Trifluoroacetyl Derivatives of Alditols and Trimethylsilyl Derivatives of Aldonolactones

Michio Matsui, Masashi Okada,[1a] Toshio Imanari, and Zenzo Tamura[1b]

Tokyo Biochemical Research Institute,[1a] and Faculty of Pharmaceutical Sciences, University of Tokyo[1b]

(Received October 2, 1967)

In connection with the studies[2,3] on the structures of oxidation products of D-glucitol (IX) and L-gulonic acid derivatives, the effective means applicable to the identification of small amounts of alditols and aldonolactones was needed. Gas chromatography was considered to meet satisfactorily the requirements. The present paper deals with the gas chromatographic separation of the trifluoroacetyl (TFA) derivatives of alditols and the trimethylsilyl (TMS) ethers of aldonolactones.

Gas chromatographic separation of alditols as their TMS ethers was found to be unsatisfactory,[4] while the separation as the acetyl derivatives gave excellent resolution.[5,6] However, the acetyl derivatives have some shortcomings. Thus rather long reaction time has been required for their preparation and, moreover, to achieve good resolution long retention time and high elution temperature have been recorded.

Recently, gas chromatography of the TFA derivatives of monosaccharides has been reported,[7,8] emphasizing their simple as well as rapid preparation and marked volatility. In view of these merits, studies on the conditions for gas chromatographic separation of the TFA derivatives of alditols were made with the expectation that they might give the similar excellent resolution to the acetates.

As shown in Table I, the TFA derivatives of eleven alditols which constitute all of the theoretically possible tetritols, pentitols, and hexitols, were separated almost within eleven

Table I. Gas Chromatography of Trifluoroacetyl Derivatives of Alditols

Alditol	Retention time	Alditol	Retention time
Erythritol (I)	0.35	D-Mannitol (VII)	1.69
L-Threitol (II)	0.45	D-Talitol (VIII)	1.90
Ribitol (III)	0.82	D-Glucitol (IX)	2.25
L-Arabinitol (IV)	1.00 (4.30 min)	L-Iditol (X)	2.25
Xylitol (V)	1.17	Galactitol (XI)	2.48
Allitol (VI)	1.49		

stationary phase: 2% CNSi column temperature: 140°

1) Location: *a) Takada 3-chome, Toshima-ku, Tokyo; b) Hongo, Bunkyo-ku, Tokyo.*
2) M.Matsui, M. Okada, and M. Ishidate, *Chem. Pharm. Bull.* (Tokyo), **16**, 1288 (1968).
3) M. Matsui, M. Saito, M. Okada, and M. Ishidate, *Chem. Pharm. Bull.* (Tokyo), **16**, 1294 (1968).
4) C. C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).
5) S.W. Gunner, J.K.N. Jones, and M.B. Perry, *Can. J. Chem.*, **39**, 1892 (1961); J.S. Sawardeker, J.H. Sloneker, and A. Jeanes, *Anal. Chem.*, **37**, 1602 (1965); E.P. Crowell and B.B. Burnett: *ibid.*, **39**, 121 (1967).
6) E. Sjöström, P. Haglund, and J. Janson, *Acta Chem. Scand.*, **20**, 1718 (1966).
7) M. Vilkas, H. Jan, G. Boussac, and M. Bonnard, *Tetrahedron Letters*, **1966**, 1441.
8) Z. Tamura and T. Imanari, *Chem. Pharm. Bull.* (Tokyo), **15**, 246 (1967).

minutes under the conditions indicated. Thus, all the alditol derivatives except those of IX and L-iditol (X) could be separated from each other.[9] A representative gas chromatogram is shown in Fig. 1.

In the determination of aldonolactones by gas chromatography, on the other hand, TMS ethers have gained a general popularity due to their easy preparation and good thermal sta-

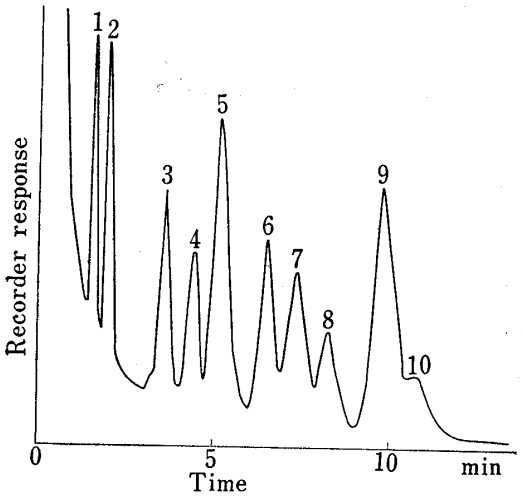

Fig. 1. Gas Chromatogram of TFA Derivatives of Alditols separated on a Column containing 2% CNSi at 140°

1) erythritol (I), 2) L-threitol (II), 3) ribitol (III), 4) L-arabinitol (IV), 5) xylitol (V), 6) allitol (VI), 7) D-mannitol (VII), 8) D-talitol (VIII), 9) D-glucitol (IX) and L-iditol (X), 10) galactitol (XI)

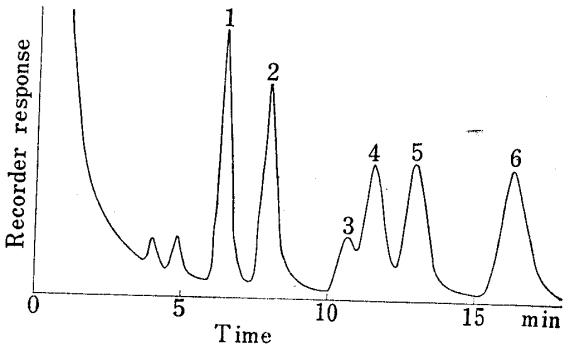

Fig. 2. Gas Chromatogram of TMS Ethers of Aldonolactones separated on a Column containing 3% NGS at 160°

1) D-altronolactone (XVIII), 2) D-galactonolactone (XXII), 3) L-idonolactone (XXIII), 4) D-talonolactone (XXV), 5) L-gulonolactone (XXVI), 6) D-mannonolactone (XX)

Table II. Retention Times of Trimethylsilyl Ethers of Aldonolactones

Aldonolactone	Stationary phase and column temperature			
	1.5% SE-52 160°	2% CNSi 170°	3% NGS 170°	3% NGS 160°
L-Threonolactone (XII)	0.12	0.09	0.21	
D-Erythronolactone (XIII)	0.16	0.14	0.38	
L-Arabonolactone (XIV)	0.38	0.26	0.34	
L-Xylonolactone (XV)	0.44	0.25	0.41	
D-Ribonolactone (XVI)	0.49	0.35	0.52	
D-Lyxonolactone (XVII)	0.61	0.47	0.79	
D-Altronolactone (XVIII)	1.12	0.63	0.54	0.51
D-Gulcono-γ-lactone (XIX)	1.20	0.74	0.70	0.68
D-Mannonolactone (XX)	1.22	0.88	1.22	1.25
D-Glucono-δ-lactone (XXI)	1.24	0.75	0.65	0.63
D-Galactonolactone (XXII)	1.27	0.74	0.64	0.62
L-Idonolactone (XXIII)	1.45	0.91	0.82	0.81
D-Allonolactone (XXIV)	1.45	0.88	0.88	0.88
D-Talonolactone (XXV)	1.54	0.98	0.88	0.89
L-Gulonolactone (XXVI)	1.00 (6.50 min)	1.00 (13.40 min)	1.00 (7.10 min)	1.00 (13.10 min)

9) Paper electrophoretic analysis of the alditols using slight modification (Toyo Roshi No. 51, 12.5×26 cm; 90 min runs at 470 V and 10—14 mA) of the procedure of Frahn, *et al.* (*Australian J. Chem.*, **12**, 65 (1959)) revealed that X showed the highest rate of migration of all the eleven alditols and was easily distinguishable from other alditols. Therefore, the complete resolution of alditols has been possible by combining both gas chromatographic and paper electrophoretic analyses.

bility.[4,6,10] In certain cases, aldonolactones have been reduced to alditols and then analysed.[6] Since an attempt to separate aldonolactones as their TFA derivatives was unsuccessful, gas chromatographic conditions for the separation of the TMS derivatives were investigated.

As shown in Table II, the TMS derivatives of fourteen aldonolactones, constituting all of the theoretically possible tetronolactones, pentonolactones, and hexonolactones, were separated almost within fifteen minutes on columns packed with several types of liquid phases. Complete resolution on a single liquid phase was quite difficult, but comparison of the retention times obtained by using different types of columns could accomplish almost complete resolution. A representative gas chromatogram of hexonolactones is shown in Fig. 2. In certain circumstances, reduction of aldonolactones to alditols and subsequent gas chromatographic analysis of their TFA derivatives should be quite effective in resolution.

From these results, systematic identification of alditols and aldonolactones has become possible.

The usual method for ascertaining the position of the carbonyl group in keto-sugars is principally based on identifying their reduction products. Thus the foregoing results on gas chromatographic analyses have been successfully applied to the identification of keto-sugars, using L-xylulose (XXVII), L-sorbose (XXVIII), 2-oxo-L-gulonic acid (XXIX) and 2,3-dioxo-L-gulonic acid (potassium salt) (XXX) as model compounds.

Treatment of XXVII with sodium borohydride gave L-arabinitol (IV) and xylitol (V) in approximately equal amount on gas chromatography as shown in Fig. 3. Reduction of XXVIII resulted in the formation of only one component with the same retention time as IX or X on gas chromatography, while paper electrophoretic analysis[9] revealed the presence of IX and X. Accordingly, the position of the carbonyl group in XXVII as well as XXVIII has been assigned to C-2. Reduction of XXIX and XXX followed by lactonization, on the other hand, indicated the presence of L-idonolactone (XXIII) and L-gulonolactone (XXVI) in the former (Fig. 4) and of D-galactonolactone (XXII) and D-talonolactone (XXV) besides XXIII and XXVI in the latter (Fig. 5) on gas chromatography. XXV and D-allonolactone (XXIV) were indistinguishable from each other on a column packed with 3% NGS (neophentyl glycol succinate), while further treatment of the mixture of these four lactones with sodium borohydride revealed the presence of galactitol (XI), D-talitol (VIII), X, and IX on gas chromatography (Fig. 6) and paper electrophoresis, thus demonstrating the absence of XXIV in the peak of XXV.

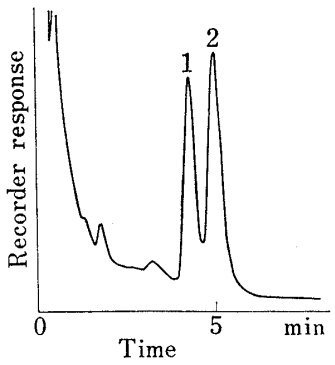

Fig. 3. Gas Chromatogram of TFA Derivatives of the Reduction Products of L-Xylulose (XXVII) separated on a Column containing 2% CNSi at 140°

1) L-arabinitol (IV), 2) xylitol (V)

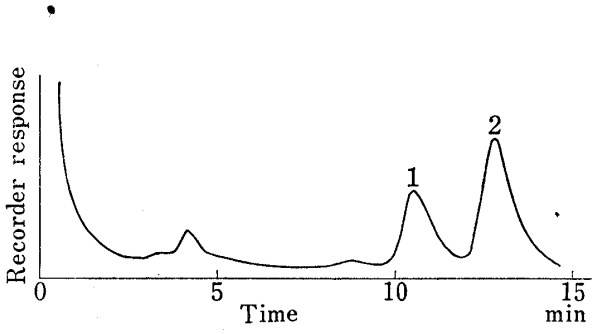

Fig. 4. Gas Chromatogram of TMS Derivatives of Aldonolactones prepared by reducing 2-Oxo-L-gulonic Acid (XXIX)

The column contained 3% NGS and was operated at 160°. 1) L-idonolactone (XXIII), 2) L-gulonolactone (XXVI)

10) M.B. Perry and R.K. Hulyalkar, *Can. J. Biochem.*, **43**, 573 (1965).

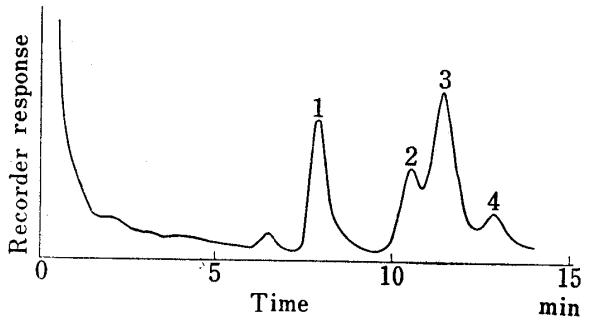

Fig. 5. Gas Chromatogram of TMS Derivatives of Aldonolactones prepared by reducing 2,3–Dioxo–L–gulonate (XXX)

The column contained 3% NGS and was operated at 160°. 1) D–galactonolactone (XXII), 2) L–idonolactone (XXIII), 3) D–talonolactone (XXV), 4) L–gulonolactone (XXVI)

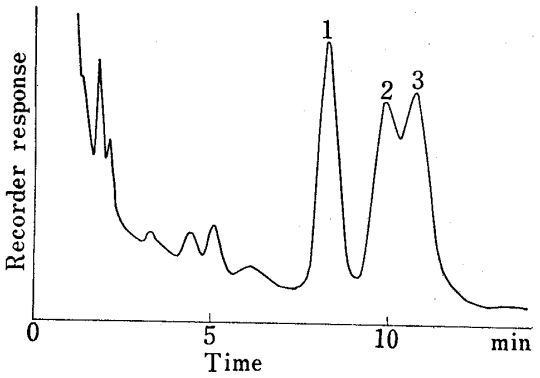

Fig. 6. Gas Chromatogram of TFA Derivatives of the Alditols prepared by reducing 2,3–Dioxo–L–gulonate (XXX)

The column contained 2% CNSi and was operated at 140°. 1) D–talitol (VIII), 2) D–glucitol (IX) and L–iditol (X), 3) galactitol (XI)

Essentially in the same way as described above, some of the results presented in this paper have been effectively applied in establishing the structures of the oxidation products of D–glucitol and L–gulonic acid derivatives.[2,3]

Experimental

Materials——D–Mannitol (VII), D–glucitol (IX), galactitol (XI), D–glucono–δ–lactone (XXI), D–galactonolactone (XXII), L–idonolactone (XXIII) (sodium salt), L–gulonolactone (XXVI) and L–sorbose (XXVIII) were commercial samples. Erythritol (I) (mp 108°) and ribitol (III) (mp 103°) were prepared from the corresponding aldoses by $NaBH_4$ reduction,[11] and L–arabinitol (IV) (mp 103°) and xylitol (V) were prepared in the same way from L–xylulose (XXVII) which was prepared from L–xylose by pyridine–catalyzed epimerization.[12] L–Threitol (II) (mp 88°), allitol (VI) (mp 150°) and D–talitol (VIII) (mp 87°) were prepared from the corresponding aldonolactones by treatment with $NaBH_4$.[13] L–Iditol (X) (mp 73°) was prepared from XXVIII by $NaBH_4$ reduction.[3] D–Erythronolactone (XIII) (mp 104°), L–arabonolactone (XIV) (mp 100°), L–xylonolactone (XV), D–ribonolactone (XVI), D–lyxonolactone (XVII) (mp 111°), D–glucono–γ–lactone (XIX) (mp 139°) and D–mannonolactone (XX) (mp 152°) were prepared from the corresponding aldoses by hypoiodite oxidation.[14] In this connection, some rare aldoses not available commercially were prepared according to the standard methods.[15] L–Threonolactone (XII) (mp 76°) was prepared by oxidizing L–ascorbic acid with $KMnO_4$.[16] D–Talonolactone (XXV) (mp 134°) was prepared from XXII by pyridine–catalyzed epimerization.[17] D–Allonolactone (XXIV) (mp 130°) and D–altronolactone (XVIII) were prepared from D–ribose by cyanohydrin synthesis.[18] 2–Oxo–L–gulonic acid (XXIX) (mp 166° (decomp.)) was prepared from sodium L–gulonate by V_2O_5–$NaClO_3$ oxidation.[19] Potassium 2,3–dioxo–L–gulonate (XXX) was prepared from L–ascorbic acid by KIO_3 oxidation.[20]

Lactonization of the aldonic acid was carried out by warming its sloution in MeOH–36% HCl (100:1) at 50° for 30 min followed by concentration of the solution under reduced pressure.[3]

11) M. L. Wolfrom and A. Thompson, "Methods in Carbohydrate Chemistry," Vol. II, ed by R. L. Whistler and M. L. Wolfrom, Academic Press, New York, **1963**, p. 65.
12) O. Touster, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York, **1962**, p. 98.
13) H.L. Frush and H.S. Isbell, *J. Am. Chem. Soc.*, **78**, 2844 (1956).
14) S. Moore and K.P. Link, *J. Biol. Chem.*, **133**, 293 (1940).
15) R.L. Whistler and M.L.Wolfrom (ed. by), "Methods in Carbohydrate Chemistry," Vol. I, Academic Press, New York, 1962.
16) J.M. Perel and P.G.Dayton, *J. Org. Chem.*, **25**, 2044 (1960).
17) C. Glatthaar and T. Reichstein, *Helv. Chim. Acta*, **21**, 3 (1938).
18) F.L. Humoller, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York, **1962**, p. 102.
19) P.P. Regna and B.P. Caldwell, *J. Am. Chem. Soc.*, **66**, 243 (1944); R. Pasternack and P.P. Regna, U.S. Patent 2188777 (1940).
20) Y. Kagawa, *J. Biochem.* (Tokyo), **51**, 134 (1962).

Gas Chromatography——Gas chromatography was performed on a Shimadzu Model GC-1B gas chromatograph equipped with a hydrogen flame ionization detector. The U-shaped stainless steel column (150 cm × 4 mm i.d.) was packed with either 3% neopentyl glycol succinate (NGS) or 2% cyanoethyl methyl silicone polymer (CNSi) on a support of Anakrom (90—100 mesh), or 1.5% methyl phenyl silicone polymer (SE-52) on a support of Gas-Chrom P (80—100 mesh). Nitrogen was used as carrier gas at a flow rate of 90 ml/min. The column operated isothermally at 140°, 160° or 170°, with an injection port temperature of 200° and a detector temperature of 190°. The TFA and the TMS derivatives of alditols and aldonolactones were prepared according to the procedures described by Sweeley, *et al.*[4] and Tamura, *et al.*,[8] respectively.

[Chem. Pharm. Bull. 16(7)1387—1390(1968)]

UDC 547.964.4.07

Studies on Peptides. XIX.[1,2] Synthesis of a Stereoisomer of α-Melanocyte-stimulating Hormone

Haruaki Yajima and Koichi Kawasaki

Faculty of Pharmaceutical Sciences, Kyoto University[3]

(Received October 9, 1967)

Recently we have reported the synthesis of an enantiomer of an active fragment of α-melanocyte-stimulating hormone (MSH), D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycine (I) and offered the first attempt to examine the physiological properties of an optical antipode of a peptide hormone.[4] We have observed that this pentapeptide (I) inhibited the action of the corresponding pentapeptide of the L-configuration, L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine (3.4×10^4 MSH U/g) in the molar ratios of 1 to 1 and the the action of α-MSH (2.0×10^{10} MSH U/g)[5] as well, but in the molar ratios of approximately 1 to 1×10^{-6}. The potency of I as an inhibitor to α-MSH is only about one millionth of that of melatonin,[6,7] which is known as the most potent, but non-specific inhibitor to α-MSH.

We have now prepared a stereoisomeric α-MSH, N^{α}-acetyl-L-seryl-L-tyrosyl-L-seryl-L-methionyl-L-glutamyl-D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-L-lysyl-L-prolyl-L-valine amide (II) in order to compare the physiological property of such a compound with that of α-MSH.

The synthetic route employed here is essentially the same as described in our synthesis of α-MSH.[8] N^{α}-Benzyloxycarbonyl-D-histidyl-D-phenylalanyl-N^{G}-nitro-D-arginyl-D-tryptophylglycine[4] was condensed with N^{ε}-formyl-L-lysyl-L-prolyl-L-valine amide[9] by dicyclohexylcarbodiimide (DCC)[10] and the resulting product was hydrogenated to give D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-N^{ε}-formyl-L-lysyl-L-prolyl-L-valine amide (III),

1) The Part XVIII of this series: *Chem. Pharm. Bull.* (Tokyo), **16**, 1342 (1968).
2) Abbreviated designation for amino acids and peptides adopted in this communication are those recommended by IUPAC-IUB commission for biological nomenclature in July 1965 and 1966: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967).
3) Location: *Sakyo-ku, Kyoto*
4) H. Yajima and K. Kubo, *J. Am. Chem. Soc.*, **87**, 2039 (1965).
5) J.I. Harris and A.B. Lerner, *Nature*, **179**, 1346 (1957).
6) A.B. Lerner, J.D. Case, and R.V. Heinzelman, *J. Am. Chem. Soc.*, **81**, 6084 (1959).
7) A.B. Lerner, J.D. Case, and Y. Takahashi, *J. Biol. Chem.*, **235**, 1992 (1960).
8) H. Yajima, K. Kawasaki, Y. Okada, H. Minami, K. Kubo, and I. Yamashita, *Chem. Pharm. Bull.* (Tokyo), **16**, 919 (1968).
9) K. Hofmann, E. Stütz, G. Spühler, H. Yajima, and E.T. Schwatrz, *J. Am. Chem. Soc.*, **82**, 3727 (1960).
10) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).