[CONTRIBUTION FROM THE CHEMICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF ZAGREB]

Optically Active Amino Aldehydes. II. Preparation of Cyclic Acetals of Quaternary Amino Aldehydes; Contribution to the Knowledge of the Stereospecificity of Muscarinic Activity[1]

K. BALENOVIĆ, N. BREGANT, T. GALIJAN, Z. ŠTEFANAC, AND V. ŠKARIĆ

Received September 28, 1955

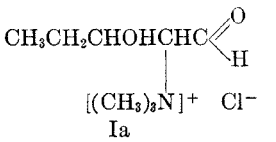
Cyclic amino acetals (Formula II) were prepared from the corresponding amino acids, or from the well crystallized tetrahydroimidazole derivatives (V) of the phthalimidoaldehydes. Quaternary ammonium salts (III) of the amino acetals (II) were prepared from L-alanine, D-alanine, α-aminobutyric acid, L-valine, DL-valine, DL-leucine, O-methyl-DL-serine, O-ethyl-DL-serine, and β-alanine. Quaternary ammonium salts derived from glycine, L-alanine and D-alanine (IIIa), and β-alanine (IV) were tested for muscarinic activity. There is no marked stereospecificity for muscarinic activity in the case of the L- and D-alanine derivative (IIIa). The quaternary ammonium salt derived from β-alanine (IV) exhibited the greatest muscarinic activity in this series of derivatives of quaternary ammonium aldehydes. The analogous compounds with free aldehyde groups or with acyclic acetal groups showed a much lower muscarinic activity.

Following Kögl,[2] muscarine should, structurally, be a formyl-hydroxybutyl-trimethylammonium compound (Ia) or a compound with the formula Ib. In his further investigations Kögl and co-workers[3] attempted to confirm the structure of muscarine by synthesizing the optically inactive compounds Ia and Ib. However, these compounds were, as compared with natural muscarine, physiologically inactive. Kögl explained this physiological inactivity of the synthetic products by assuming that the muscarine molecule is extremely stereospecific, and that only inactive stereoisomers were obtained by synthesis.

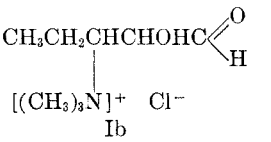
Recently, Eugster and Waser[4] proposed the formula $C_9H_{20}O_2N^+$ for muscarine, and found that muscarine is a completely saturated compound without ethylenic double bonds or carbonyl groups.

It is evident, therefore, that muscarine contains a ring. Under the circumstances, it seemed to be of considerable interest to begin a systematic investigation of this alkaloid, as much from the analytical as from the synthetic point of view. In this paper a description will be given of the preparation of optically active cyclic acetals of quaternary amino aldehydes and the results of our investigations on the muscarinic activity of these compounds will be summarized.

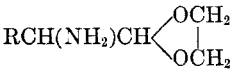
We have already shown[5] that N-phthaloyl derivatives of optically active α-amino acids are particularly convenient starting materials for these studies. The Rosenmund-Zetzsche reduction of N-phthaloyl acyl chlorides gave optically active N-phthaloyl amino aldehydes, which after splitting off the phthaloyl group gave cyclic acetals (II) with free amino groups. Subsequent alkylation of the amino group with methyl iodide and sodium hydroxide in methanol gave optically active quaternary salts of ammonium acetals (III).



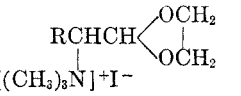
Ia



Ib



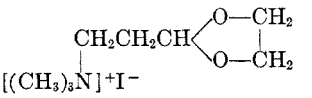
II



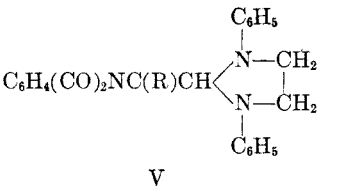
III

a, R = CH_3
b, R = C_2H_5
c, R = $(CH_3)_2CH$

d, R = $(CH_3)_2CHCH_2$
e, R = CH_3OCH_2
f, R = $C_2H_5OCH_2$



IV



V

Quaternary ammonium salts (III) of amino acetals (II) were prepared from L-alanine (a), D-alanine, α-aminobutyric acid (b), L-valine (c), DL-valine, DL-leucine (d), O-methyl-DL-serine (e), O-ethyl-DL-serine (f) and β-alanine (IV).

The corresponding quaternary ammonium salt of glycine (III, R=H) was prepared by Fourneau and co-workers[6] some years ago, from betaine aldehyde, and it showed a comparatively high muscarinic activity.

We have investigated the muscarinic activity of the above mentioned quaternary ammonium salts

(1) A part of this paper was presented at the XIVth International Congress of Pure and Applied Chemistry, Zürich, July 1955.

(2) Kögl, Duisberg, and Erxleben, *Ann.*, **489**, 156 (1931).

(3) Kögl and Veldstra, *Ann.*, **552**, 1 (1942).

(4) Eugster and Waser, *Experientia*, **10**, 298 (1954).

(5) Balenović, Bregant, Cerar, Fleš, and Jambrešić, *J. Org. Chem.*, **18**, 297 (1953).

(6) E. Fourneau, Bovet, Montezin, J. P. Fourneau, and Chantalou, *Ann. pharm. franç.*, **3**, 114 (1945).

III using Führer's method[7] for the measuring of muscarinic activity on isolated frog hearts, and by estimating the muscarine units according to Kögl.[2] The most interesting results are given in Table I.

TABLE I

MUSCARINIC ACTIVITY OF QUATERNARY AMMONIUM SALTS

Quaternary ammonium salt	Parent compound	Muscarinic activity for 1 g. of substance[a]
III, R = H	Glycine	78,000 M.U.[b]
IIIa	L-Alanine	110,000 M.U.
	D-Alanine	170,000 M.U.
IV	β-Alanine	340,000 M.U.
Natural muscarine		200×10^6 M.U.[c]
		238×10^6 M.U.[d]

[a] Muscarine units according to Kögl.[2] [b] Our determination of the muscarinic activity of the compound first prepared by Fourneau, *et al.*[6] [c] Determinations carried out by Kögl, *et al.*[2] on *Rana esculenta*. [d] Determinations carried out by Eugster and Waser[4] on *Rana temporaria*.

It is interesting that the quaternary ammonium salts of cyclic amino acetals (III) exhibit a greater muscarinic activity than do the corresponding quaternary ammonium aldehydes. Betaine aldehyde, for instance, is much less active than Fourneau's compound (III, R=H). It appears that our β-alanine derivative is the hitherto most active synthetic compound derived from ammonium aldehydes which shows muscarinic activity.

We also prepared some aliphatic acetals of betaine aldehyde, but they were completely inactive. We have tested the muscarinic activity of the other quaternary salts of the type III, but the activity was not higher than of those given in Table I, and the results will be published elsewhere.

Acknowledgment. We are indebted to the Fund for Scientific Research of the University of Zagreb for financial assistance during 1954. We also wish to express our thanks to Drs. I. Jambrešič and D. Keglević for some of the starting materials.

TABLE II

THE PHTHALOLYL ALDEHYDES

Compound	Empirical Formula	Yield, %	M.P., °C.	Analyses Calc'd C	Calc'd H	Found C	Found H
α-Phthalimidobutyraldehyde	$C_{12}H_{11}NO_3$	62	106–108[a]	66.35	5.10	66.62	5.22
N-Phthaloyl-DL-valine aldehyde	$C_{13}H_{13}NO_3$	61	60[a]	67.52	5.67	67.62	5.63
N-Phthaloyl-DL-leucine aldehyde	$C_{14}H_{15}NO_3$	87	60[b]	68.55	6.16	68.50	6.24

[a] Colorless prisms, recrystallized from dichloromethane-petroleum ether. [b] Colorless prisms, recrystallized from benzene petroleum ether.

TABLE III

DERIVATIVES OF PHTHALOLYL ALDEHYDES

Parent compound	Derivative	Empirical Formula	M.P., °C.	Analyses Calc'd C	Calc'd H	Found C	Found H
N-Phthaloyl-L-valine aldehyde[a]	Semicarbazone[b]	$C_{14}H_{16}N_4O_3$	223	58.32	5.59	58.61	5.59
	2,4-Dinitrophenylhydrazone[c]	$C_{19}H_{17}N_5O_6$	133–134	55.47	4.16	55.57	4.25
	Tetrahydroimidazole derivative (Vc)[d]	$C_{27}H_{27}N_3O_2$	130–131	76.21	6.40	76.36	6.71
N-Phthaloyl-L-leucine aldehyde[e]	Semicarbazone[b]	$C_{15}H_{18}N_4O_3$	182–183	59.59	6.00	59.59	6.26
	2,4-Dinitrophenylhydrazone[c]	$C_{20}H_{19}N_3O_6$	165–167	56.46	4.50	57.09	4.81
	Diethyl acetal[f]	$C_{18}H_{25}NO_4$		67.68	7.89	68.22	7.60
O-Methyl-N-phthaloyl-DL-serine aldehyde[g]	Tetrahydroimidazole derivative (Ve)[c]	$C_{26}H_{25}N_3O_3$	154.5	73.05	5.90	73.35	5.87
O-Ethyl-N-phthaloyl-DL-serine aldehyde[h]	Semicarbazone[i]	$C_{14}H_{16}N_4O_4$	196.5	55.26	5.30	55.70	5.09

[a] Yield 95% of pale yellow oil, $[\alpha]_D^{23}$ −47.3° ± 1° (*c*, 5.4 in benzene). [b] Colorless needles from methanol. [c] Yellow needles from ethanol. [d] Yellow needles from methanol, $[\alpha]_D^{21}$ −29.1° ± 0.6° (*c*, 0.55 in benzene); [e] Yield, 80% of pale yellow oil, $[\alpha]_D^{19}$ −40.2° ± 2° (*c*, 5.17 in benzene). [f] Pale yellow oil, b.p. 130–135°/0.03 mm., $[\alpha]_D^{20}$ −15.2° ± 0.3° (*c*, 3.62 in benzene). [g] Yield, 49% of pale yellow oil. [h] Pale yellow oil, yield 28.4%. [i] White prisms from methanol.

These results show that the optical antipodes of the quaternary ammonium salts IIIa, derived from L- and D-alanine respectively, show no important differences in muscarinic activity.

(7) Führer in *Abderhalden's Handbuch d. biol. Arbeitsmethoden*, **Abt. IV., Teil 7**, Erste Hälfte, pp. 530–565.

EXPERIMENTAL

All melting points are uncorrected.

α-Phthalimido aldehydes were prepared according to the procedure described by Balenović, *et al.*[5]

In several cases where isolation of N-phthaloyl aldehydes from oily reaction products was difficult, well crystallized condensation products (V) with N,N-diphenyl-1,2-diamino-

ethane[8] (*vide infra*) were obtained, from which the pure N-phthaloyl aldehyde could be regenerated by refluxing with an equimolar quantity of *p*-toluenesulfonic acid in benzene solution.

1,3-Diphenyl-2-(2-phthalimidoethyl)tetrahydroimidazole was obtained from crude β-phthalimidopropionaldehyde prepared by the Rosenmund-Zetzsche reduction of β-phthalimidopropionyl chloride,[9] and N,N-diphenyl-1,2-diaminoethane. Colorless needles, recrystallized from methanol, m.p. 148°.

Anal. Calc'd for $C_{25}H_{23}N_3O_2$ (397.46): C, 75.54; H, 5.83. Found: C, 75.90; H, 5.72.

PREPARATION OF AMINOACETALS. II

N-Phthaloyl-L-alanine aldehyde ethylene acetal (IIa). A mixture of N-phthaloyl-L-alanine aldehyde (6.1 g., 0.03 mole, $[\alpha]_D$ −26°), ethanediol (7.5 ml.), *p*-toluenesulfonic acid (0.2 g.), and benzene (300 ml.), was slowly distilled from a flask provided with a total condensation take-off adapter. After five hours 1.2 ml. of water had distilled. The mixture was cooled, washed with water, dried (Na_2SO_4), and evaporated to dryness. The crystalline residue of N-phthaloyl-L-alanine aldehyde ethylene acetal, yield 7.2 g. (96%), m.p. 93°, was recrystallized from dichloromethane-petroleum ether (1:1), m.p. unchanged.

In the same manner N-phthaloyl derivatives of the amino acetals IIb-e were prepared. Additional data are given in Table IV.

In several cases N-phthaloyl aldehyde ethylene acetals were very conveniently prepared in the above mentioned manner, but starting from the condensation product of the N-phthaloyl aldehyde with N,N-diphenyl-1,2-diaminoethane (V) and equimolar quantities of *p*-toluenesulfonic acid.

β-Phthalimidopropionaldehyde ethylene acetal from 1,3-diphenyl-2-(2-phthalimidoethyl)tetrahydroimidazole. A mixture of 1,3-diphenyl-2-(2-phthalimidoethyl)tetrahydroimidazole (20 g., 0.05 mole), ethanediol (12.5 ml.), and benzenesulfonic acid (15.8 g., 0.1 mole), in benzene (900 ml.) and water (10 ml.) was slowly distilled from a flask provided with a total condensation take-off adapter. After 8 hours the reaction mixture was cooled, filtered, washed with water, and the benzene layer was dried (Na_2SO_4). After removing the benzene by distillation, crude β-phthalimidopropionaldehyde ethylene acetal, yield 12 g., (100%), m.p. 86–100° was obtained. Recrystallization from dichloromethane-petroleum ether gave colorless needles of the pure product, yield 6.2 g. (50%), m.p. 113–115°.

Anal. Calc'd for $C_{13}H_{13}NO_4$ (247.24): C, 63.15; H, 5.30. Found: C, 63.31; H, 5.25.

Hydrazinolysis of the N-phthaloyl derivatives of amino acetals to the free amino acetals (II) was carried out with an 1 *M* ethanolic hydrazine hydrate solution. The data for amino acetals obtained in this manner are summarized in Table V and for their picrates in Table VI. As an example the following preparation is given.

β-Aminopropionaldehyde ethylene acetal. A mixture of β-phthalimidopropionaldehyde ethylene acetal (8 g., 0.03 mole), an ethanolic 1 *M* solution of hydrazine hydrate (75 ml., 0.035 mole), and ethanol (60 ml.) was refluxed for three hours. After cooling the reaction mixture, the phthalyl hydrazide was filtered off, dichloromethane was added to the filtrate, and an additional quantity of phthalyl hydrazide was removed (total phthalyl hydrazide 75%). The filtrate

TABLE IV

N-PHTHALOYL DERIVATIVES OF AMINO ACETALS IIa–f

Parent compound	M.p., °C.	$[\alpha]_D$	Formula	Analyses Calc'd C	Calc'd H	Found C	Found H
L-Alanine	93[a]	$[\alpha]_D^{22}$ +23.0° ± 0.2° (*c*, 2.50 in benzene)	$C_{13}H_{13}NO_4$	63.15	5.30	63.25	5.25
D-Alanine	95[a]	$[\alpha]_D^{22}$ −21.0° ± 0.3° (*c*, 2.85 in benzene)	$C_{13}H_{13}NO_4$	63.15	5.30	62.88	5.40
DL-α-Aminobutyric acid	60–62[b]		$C_{14}H_{15}NO_4$	64.38	5.75	64.23	5.71
DL-Valine	81[a]		$C_{15}H_{17}NO_4$	65.44	6.23	65.27	6.17
L-Valine	30[a]	$[\alpha]_D^{22}$ −4.7° ± 0.1° (*c*, 7.07 in benzene)	$C_{15}H_{17}NO_4$	65.44	6.23	65.24	6.26
DL-Leucine	[c]		$C_{16}H_{19}NO_4$	66.42	6.62	66.03	6.43
O-Ethyl-DL-serine	54[d]		$C_{15}H_{17}NO_5$	61.85	5.88	62.13	5.80

[a] Recrystallized from dichloromethane-petroleum ether, colorless prisms. [b] Recrystallized from petroleum ether, colorless platelets. [c] Yellow oil, b.p. 125–130°/0.03 mm. [d] B.p. 125–130°/0.004 mm.

TABLE V

AMINO ACETALS OF THE FORMULA IIa–f

Parent compound	B.p., °C.	Mm.	$[\alpha]_D$	Formula	Analyses Calc'd C	Calc'd H	Found C	Found H
L-Alanine	65–75	18	$[\alpha]_D^{20}$ +16.3° ± 1.1° (*c*, 1.32 in n/10 HCl)	$C_5H_{11}NO_2$	51.26	9.47	51.14	9.39
D-Alanine	65–70	18	$[\alpha]_D^{20}$ −15.9° ± 0.3° (*c*, 1.59 in n/10 HCl)	$C_5H_{11}NO_2$	51.26	9.47	51.12	9.56
DL-α-Aminobutyric acid	70–71	18		$C_6H_{13}NO_2$	54.94	9.99	54.48	10.21
DL-Valine	80–85	12		$C_7H_{15}NO_2$	57.90	10.41	57.66	10.26
DL-Leucine	75–80	15		$C_8H_{17}NO_2$	60.34	10.76	60.60	10.71
O-Ethyl-DL-serine	95–100	15		$C_7H_{15}NO_3$	52.15	9.38	51.70	9.24

(8) Wanzlick and Löchel, *Chem. Ber.*, **86**, 1463 (1953); cf. Billman, Ju Yu Chen Ho, and Caswell, *J. Org. Chem.*, **17**, 1375 (1952).

(9) Jambrešić and Sunko, *Arhiv kem.*, **23**, 195 (1951).

TABLE VI

PICRATES OF AMINOACETALS IIa–f

Parent compound	M.p., °C.	Formula	Analyses Calc'd C	Calc'd H	Found C	Found H
L-Alanine	207[a]	$C_{11}H_{14}N_4O_9$	38.15	4.08	37.92	4.00
D-Alanine	210[a]	$C_{11}H_{14}N_4O_9$	38.15	4.08	38.52	4.24
DL-α-Aminobutyric acid	201[b]	$C_{12}H_{16}N_4O_9$	40.00	4.48	39.68	4.44
DL-Valine	184[b]	$C_{13}H_{18}N_4O_9$	41.71	4.85	41.90	4.83
L-Valine	186[b]	$C_{13}H_{18}N_4O_9$	41.71	4.85	41.68	4.88
DL-Leucine	169–170[b]	$C_{14}H_{20}N_4O_9$	43.30	5.19	43.41	5.14
O-Methyl-DL-serine	214[c]	$C_{12}H_{16}N_4O_{10}$	38.30	4.29	38.53	4.36
O-Ethyl-DL-serine	150[b]	$C_{13}H_{18}N_4O_{10}$	40.00	4.65	40.14	4.66

[a] Recrystallized from methanol, yellow prisms. [b] Recrystallized from ethyl acetate-petroleum ether, yellow needles. [c] Recrystallized from ethyl acetate, yellow needles.

TABLE VII

QUATERNARY SALTS OF THE FORMULA IIIa–f

Parent compound	M.p., °C.	$[\alpha]_D$	Formula	Analyses Calc'd C	Calc'd H	Found C	Found H
L-Alanine	221–222[a]	$[\alpha]_D^{18}$ −15.2° ± 0.4° (*c*, 1.22 in water)	$C_8H_{18}INO_2$	33.46	6.32	33.55	6.25
D-Alanine	219–220[b]	$[\alpha]_D^{18}$ +14.1° ± 0.2° (*c*, 1.03 in water)	$C_8H_{18}INO_2$	33.46	6.32	33.07	6.43
DL-α-Aminobutyric acid	187[a]		$C_9H_{20}INO_2$	35.89	6.69	35.79	6.63
DL-Valine	160–161[c]		$C_{10}H_{22}INO_2$	38.10	7.04	37.75	6.84
L-Valine	184–185[d]	$[\alpha]_D^{24}$ +10.5° ± 0.8° (*c*, 2.37 in water)	$C_{10}H_{22}INO_2$	38.10	7.04	38.02	7.13
DL-Leucine	191[a]		$C_{11}H_{24}INO_2$	40.13	7.35	39.95	7.44
O-Methyl-DL-serine	181–182[a]		$C_9H_{20}INO_3$	34.08	6.36	33.95	6.48
O-Ethyl-DL-serine	144[e]		$C_{10}H_{22}INO_3$	36.26	6.70	36.10	6.72

[a] Recrystallized from absolute ethanol-petroleum ether, colorless needles. [b] Recrystallized from absolute ethanol, colorless needles. [c] Recrystallized from acetone, colorless needles. [d] Recrystallized from absolute ethanol-ether, colorless needles. [e] Recrystallized from absolute ethanol-ether, colorless prisms.

was evaporated, and the β-aminopropionaldehyde ethylene acetal was distilled at 70–75°/18 mm.

Anal. Calc'd for $C_5H_{11}NO_2$ (117.15): C, 51.26; H, 9.47. Found: C, 51.07; H, 9.47.

Preparation of the quaternary ammonium salts of amino acetals IIa–f was carried out following Fischer's procedure for the preparation of betaine aldehyde diethyl acetal.[10] In our experiments sodium hydroxide was used instead of potassium hydroxide.

(1-Formylethyl)trimethylammonium iodide ethylene acetal (IIIa) was obtained from L-alanine aldehyde ethylene acetal (IIIa) was obtained from L-alanine aldehyde ethylene acetal (IIa) as colorless needles, yield 80.7%, m.p. 221–222°. Additional data for this and other quaternary ammonium salts will be found in Table VII.

(10) Fischer, *Ber.*, **26**, 464 (1893).

(2-Formylethyl)trimethylammonium iodide ethylene acetal (IV) was obtained from β-aminopropionaldehyde ethylene acetal also by Fischer's procedure. On cooling, 22% of (2-formylethyl)trimethylammonium iodide ethylene acetal separated. The mother liquor therefore was evaporated, and the residue was dissolved in absolute ethanol. From this solution, an additional quantity of pure product could be obtained by precipitation with ether. Total yield of colorless platelets 68%, m.p. 235°.

Anal. Calc'd for $C_8H_{18}INO_2$ (287.15): N, 4.88. Found: N, 5.15.

The *biological test* was carried out for each compound on 20 frog hearts (*Rana esculenta*) following Kögl's technique.[2] The error of the method was ±15%.

STROSSMAYEROV TRG 14
ZAGREB, YUGOSLAVIA