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Semisynthetic Penicillins

IV.* Preparation of α-(Ylideneimino-oxy)carboxylic Acids

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The synthesis of Schiff bases, obtained by reacting α-amino-oxy carboxylic acids with aldehydes and ketones, is described. One of the compounds prepared, *viz.* α-(2-chlorobenzylideneimino-oxy)phenylacetic acid, has been resolved into its optical antipodes and its configuration related to that of mandelic acid.

Some naturally occurring amino-oxy compounds, *e.g.* canavanine[1,2] (α-amino-γ-guanidino-oxybutyric acid) and cycloserine[3,4] (D-4-amino-3-isoxazolidone), as well as some amino-oxycarboxylic acids and N-substituted derivatives thereof have been shown to possess antibacterial activity. McHale *et al.*[5] reported growth inhibitory effects for a series of aliphatic α-amino-oxycarboxylic acids against *Staphylococcus aureus* Oxford, *Escherichia coli* and *Mycobacterium tuberculosis*. Testa *et al.*[6] prepared the amino-oxy analogues of naurally occurring α-amino acids and found various members to exhibit growth inhibitory effects against *inter alia Staph. aureus*, *E. coli* and *M. tuberculosis*.

In connection with our penicillin studies, we decided to investigate whether introduction of an amino-oxy moiety into the side chain of a penicillin would enhance the biological activity against Gram-negative bacteria. With the exception of N-benzoyl- and N,N-dibenzyloxycarbonylamino-oxypenicillins which have been described in the patent literature[7] as intermediates in the synthesis of hydroxypenicillins, the preparation of this type of compounds does not appear to have been reported.

In the present paper, we report the preparation of a series of N-substituted α-amino-oxycarboxylic acids, used in the syntheses of the penicillins to be described later.** Nicolaus *et al.*[8] and Schumann *et al.*[9] have reviewed the different routes leading to O-substituted hydroxylamines. In accordance with

* Paper III, B. Ekström, A. Goméz-Revilla, R. Mollberg, H. Thelin and B. Sjöberg, *Acta Chem. Scand.* **19** (1965) 281.

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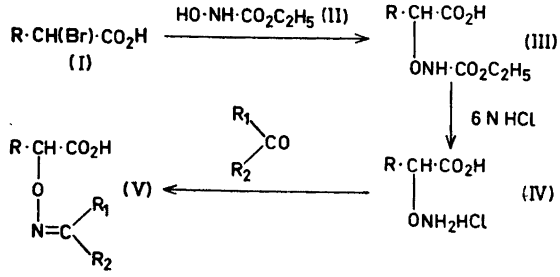
these authors, we found the N-hydroxyurethane method (Scheme I) to be the most suitable for the preparation of amino-oxy acids. α-Bromocarboxylic acids (I) — commercially available or prepared by standard methods — were reacted with N-hydroxyurethane (II) using sodium ethoxide in ethanol as condensing agent. The resulting α-(N-carbethoxyamino-oxy)carboxylic acids (III) were hydrolyzed with hot 6 N hydrochloric acid to yield the corresponding α-amino-oxycarboxylic acid hydrochlorides (IV). These reacted readily with various aldehydes and ketones under weakly alkaline conditions to yield the corresponding Schiff bases (V).



Scheme 1

The Schiff bases were often difficult to crystallize and therefore the carbonyl compounds used as starting materials were thoroughly purified by distillation or recrystallization.

The prepared α-(N-carbethoxyamino-oxy)carboxylic acids (III), the α-amino-oxycarboxylic acids (IV) and the Schiff bases (V) are listed in Tables 1—3.

Table 1. α-(N-Carbethoxyamino-oxy)carboxylic acids.

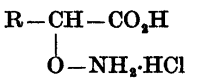
R—CH—CO_2H
|
ONHCOOC_2H_5 (III)

No.	R	Formula	M.p.	Yield %		Analysis			
IIIa	C_6H_5	$C_{11}H_{13}NO_5$	117—9	67	found	C 55.4	H 5.45	N 5.75	O 33.1
					calc.	55.2	5.48	5.86	33.4
IIIb	2-ClC_6H_4	$C_{11}H_{12}NO_5Cl$	130—2	63	found	C 48.0	H 4.53	N 4.70	
					calc.	48.3	4.39	5.12	
IIIc	4-ClC_6H_4	$C_{11}H_{12}NO_5Cl$	134—9	61	found	C 48.3	H 4.44		O 28.8
					calc.	48.3	4.39		29.3
IIId[18]	3-thienyl	$C_9H_{11}NO_5S$	113—5	66	found	C 44.0	H 4.52	N 5.78	S 13.1
					calc.	44.1	4.49	5.21	13.1
IIIe	CH_3	$C_6H_{11}NO_5$	96—7	63	found	C 40.9	H 6.35	N 7.76	O 45.2
					calc.	40.7	6.26	7.91	45.2

Of the penicillins prepared from the compounds listed in Table 3, α-(2-chlorobenzylideneimino-oxy)benzylpenicillin — synthesized from acid Vd, Table 3 — showed the best activity against Gram-negative bacteria. We have previously shown that in the case of α-aminobenzylpenicillin, a marked difference of activity exists between the epimeric forms obtained by coupling D- or L-phenylglycin with 6-aminopenicillanic acid.[10] We therefore decided to prepare the two epimeric penicillins originating from the D- and L-forms of the acid Vd (Table 3). Fractional crystallization of the (+)-ephedrine salt of Vd from acetone gave the levorotatory isomer. The dextrorotatory form was obtained from the (+)-phenylisopropylamine salt in methanol.

In order to relate the stereochemistry of Vd to a compound of known absolute configuration, two ways were considered. Quasiracemate formation with *e.g.*, O-benzyl- or O-benzoylmandelic acid appeared to be promising. However, it is known from the patent literature that α-(N-dibenzyloxycarbonyl-amino-oxy)phenylacetic acid (VI, Scheme II) is easily hydrogenolyzed[11] to form mandelic acid. By the latter way we related the configuration of acid Vd to mandelic acid. Racemic α-amino-oxyphenylacetic acid was reacted with benzyloxycarbonyl chloride yielding α-(N-benzyloxycarbonylamino-oxy) phenylacetic acid (VI). Fractional crystallization of the (+)-phenylisopropylamine salt of VI yielded the (−)-isomer. From the latter, optically pure (−)-mandelic acid was obtained by hydrogenolysis. As it has been conclusively shown that (−)-mandelic acid belongs to the D-series[12–14] the absolute configuration of levorotatory VI is thus determined. D-(−)-VI was hydrolyzed to D(−)-IVa which was reacted with 2-chlorobenzaldehyde to yield (−)-Vd (Scheme II). Also to this isomer the D-configuration must be ascribed as the hydrolysis of the benzyloxycarbonyl compound VI does not affect the asymmetric centre.

Table 2. α-Amino-oxycarboxylic acid hydrochlorides.



(IV)

No.	R	Formula	M.p.	Yield %
IVa	C_6H_5	$C_8H_{10}NO_3Cl$	178–180°	88
IVb	2-ClC_6H_4	$C_8H_9NO_3Cl_2$	165–170°	71
IVc	4-ClC_6H_4	$C_8H_9NO_3Cl_2$	170–174°	85
IVd	3-thienyl	$C_6H_8NO_3ClS$	> 150 dec.	65
IVe	CH_3	$C_3H_8NO_3Cl$	163–164°	70

The Schiff base D(−)Vd obtained in this manner had an $[\alpha]_D^{20}$ of −94.7° (ethanol), which is 0.8° less than that found by direct resolution of racemic Vd. These two products showed identical IR-spectra and gave no mixed melting point depression.

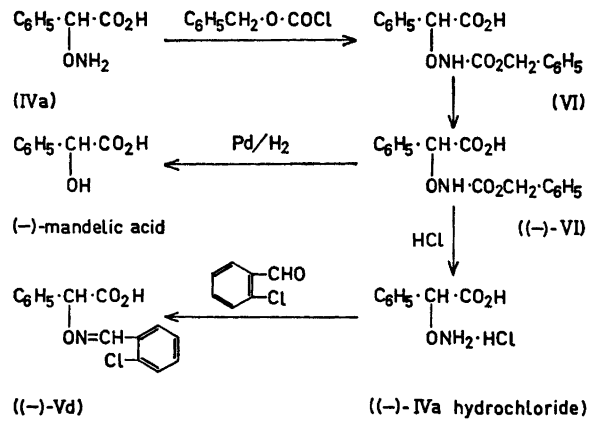
IR-spectra in KBr of racemic Vd and of its antipodes showed distinct differences, especially in the carbonyl absorption region (Fig. 1). The strong

Table 3. Schiff bases.

$$R-CH(O-N=R')-CO_2H$$

No.	R	R′	M.p.	Formula	Yield %	Analysis				
Va	CH_3	CH-2,6-$Cl_2C_6H_3$	98–100	$C_{10}H_9NO_3Cl_2$	72	found	C 45.9	H 3.62	N 4.98	Cl 26.95
						calc.	45.8	3.43	5.35	27.1
Vb	C_6H_5	CH-C_6H_5	103–5	$C_{15}H_{13}NO_3$	70	found	C 70.4	H 5.13	N 5.44	O 18.8
						calc.	70.6	5.09	5.49	18.8
Vc	C_6H_5	CH-2-FC_6H_4	91–3	$C_{15}H_{12}NO_3F$	51	found	C 66.0	H 4.50	N 5.41	F 6.77
						calc.	65.9	4.39	5.13	6.96
Vd	C_6H_5	CH-2-ClC_6H_4	104–5	$C_{15}H_{12}NO_3Cl$	80	found	C 62.4	H 4.36	N 4.85	Cl 12.1
						calc.	62.2	4.17	4.85	12.2
Ve	C_6H_5	CH-2-BrC_6H_4	125–7	$C_{15}H_{12}NO_3Br$	94	found	C 53.9	H 3.60	N 4.24	Br 24.1
						calc.	53.9	3.62	4.19	23.9
Vf	C_6H_5	CH-3-ClC_6H_4	107–9	$C_{15}H_{12}NO_3Cl$	74	found	C 61.8	H 3.96	N 5.02	Cl 12.0
						calc.	62.2	4.17	4.85	12.2
Vg	C_6H_5	CH-4-ClC_6H_4	167–8	$C_{15}H_{12}NO_3Cl$	32	found	Cl 12.2	N 4.99	O 16.3	
						calc.	12.2	4.85	16.57	
Vh	C_6H_5	CH-2,4-$Cl_2C_6H_3$	176–7	$C_{15}H_{11}NO_3Cl_2$	94	found	C 55.4	H 3.83	N 4.38	Cl 21.6
						calc.	55.6	3.42	4.32	21.9
Vi	C_6H_5	CH-2,6-$Cl_2C_6H_3$	85–90	$C_{15}H_{11}NO_3Cl_2$	59	found	C 56.0	H 3.59	N 4.05	
						calc.	55.6	3.42	4.32	
Vj	C_6H_5	CH-2-HOC_6H_4	111–3	$C_{15}H_{13}NO_4$	72	found	C 66.3	H 4.89	N 5.34	
						calc.	66.4	4.80	5.16	
Vk	C_6H_5	CH-2-$CH_3C_6H_4$	121–2	$C_{16}H_{15}NO_3$	79	found	C 71.3	H 5.65	N 5.30	
						calc.	71.4	5.58	5.21	
Vl	C_6H_5	CH-2-$CH_3OC_6H_4$	103–4	$C_{16}H_{15}NO_4$	68	found	C 67.2	H 5.42	N 5.00	
						calc.	67.4	5.26	4.91	

Vm	C_6H_5	CH-4-$NO_2C_6H_4$	203–4	$C_{15}H_{12}N_2O_5$	22	found	C 60.0	H 4.01	N 9.49	O 26.7			
						calc.	60.0	4.03	9.33	26.6			
Vn	C_6H_5	CH-4-$(CH_3)_2N$-C_6H_4	147–8	$C_{17}H_{18}N_2O_3$	93	found	C 68.1	H 5.75	N 9.41	O 16.1			
						calc.	68.4	6.08	9.39	16.1			
Vo	C_6H_5	CH-2-HO-5-ClC_6H_3	133–8	$C_{15}H_{12}NO_4Cl$	75	found	C 59.4	H 4.21	N 4.62	O 21.1	Cl 10.8		
						calc.	58.9	3.93	4.58	21.0	11.6		
Vp	C_6H_5	$CHCCl_3$	107–9	$C_{10}H_8NO_3Cl_3$	52	found	C 40.8	H 2.85	O 16.1	Cl 35.0			
						calc.	40.4	2.70	16.2	35.9			
Vq	C_6H_5	$C(CH_3)_2$	103–8	$C_{11}H_{13}NO_3$	50	found	C 63.6	H 6.31	N 6.67	O 23.5			
						calc.	63.7	6.28	6.76	23.2			
Vr	C_6H_5	cyclohexylidene	121–3	$C_{14}H_{17}NO_3$	60	found	C 68.0	H 7.00	N 5.53				
						calc.	68.0	6.88	5.66				
Vs	C_6H_5	$C(CH_2Cl)CH_3$	88–91	$C_{11}H_{13}NO_3Cl$	65	found	C 54.8	H 5.11	N 5.67	O 19.9	Cl 14.6		
						calc.	54.3	5.37	5.80	19.9	14.7		
Vt	C_6H_5	$C(C_6H_5)_2$	182–3	$C_{21}H_{17}NO_3$	31	found	C 76.5	H 5.36	N 4.22	O 14.5			
						calc.	76.1	5.17	4.23	14.5			
Vu	C_6H_5	2-HO-naphthylidene-1	125–6	$C_{19}H_{15}NO_4$	83	found	C 71.1	H 4.49	N 4.32	O 19.8			
						calc.	71.4	4.71	4.36	19.9			
Vv	C_6H_5	furfurylidene-2	122–4	$C_{13}H_{11}NO_4$	68	found	C 63.5	H 4.55	N 5.93	O 25.9			
						calc.	63.6	4.49	5.71	26.1			
Vw	C_6H_5	thienylidene-2	88–90	$C_{13}H_{11}NO_3S$	67	found	C 58.8	H 4.28	N 5.22				
						calc.	59.8	4.21	5.36				
Vx	C_6H_5	thienylidene-3	115–8	$C_{13}H_{11}NO_3S$	41	found	C 60.0	H 4.34	N 5.36	S 12.33			
						calc.	59.8	4.21	5.36	12.35			
Vy	C_6H_5	4-Br-thienylidene-3	109–11	$C_{13}H_{10}NO_3BrS$	43	found	C 46.2	H 3.07	N 3.99	S 9.25	Br 23.8		
						calc.	45.9	2.94	4.12	9.40	23.5		
Vz	C_6H_5	3-Br-thienylidene-2	91–4	$C_{13}H_{10}NO_3BrS$	40	found	equivalent weight: 344			Br 23.3			
						calc.			340	23.5			
Vaa	3-thienyl	CH-2-Cl-C_6H_4	131–5	$C_{13}H_{10}NO_3ClS$	50	found	C 52.7	H 3.53	N 4.67	S 10.81			
						calc.	52.8	3.38	4.74	10.83			
Vab	2-Cl-C_6H_4	CH-2-Cl-C_6H_4	127–32	$C_{15}H_{11}NO_3Cl_2$	56	found	C 55.5	H 3.55	N 4.14	O 14.9	Cl 21.6		
						calc.	55.6	3.40	4.30	14.8	21.9		
Vac	4-Cl-C_6H_4	CH-2-Cl-C_6H_4	144–7	$C_{15}H_{11}NO_3Cl_2$	74	found	C 56.0	H 3.83	N 3.99	O 15.1	Cl 21.3		
						calc.	55.6	3.40	4.30	14.8	21.9		



Scheme II

carbonyl absorption at 1725 cm^{-1} of the racemic compound was for the D or L acid split into two bands situated at 1670 cm^{-1} and 1705 cm^{-1}. In carbon tetrachloride, these differences disappear and identical spectra are obtained. This behaviour may be interpreted as a crystal effect, as reported previously for racemic forms and their antipodes.[15-17]

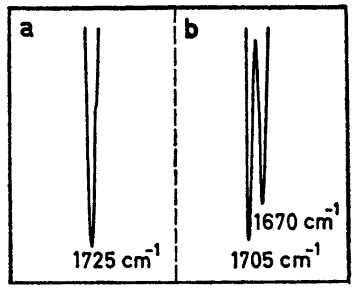


Fig. 1. Carbonyl region of the infrared absorption spectra in KBr of racemic (a) and D(−)-form (b) of α-(2-chlorobenzylideneimino-oxy)phenylacetic acid (Vd).

EXPERIMENTAL

Melting points were determined on a copper block, using calibrated thermometers. Optical rotations were measured on a polarimeter, type Perkin-Elmer 141.

α-(N-Carbethoxyamino-oxy)phenylacetic acid (IIIa, Table 1). α-Bromophenylacetic acid (21.5 g, 0.1 mole) was added in small portions at 0°C to a stirred solution of sodium (5.3 g, 0.23 mole) and hydroxyurethane (13.65 g, 0.13 mole) in 200 ml ethanol. The mixture was refluxed for 5 h, the ethanol evaporated *in vacuo* and the residue dissolved in 300 ml ice-cold water. After extraction with ether, the aqueous phase was acidified to pH 2—2.5 with HCl and the oily precipitate extracted with ether. After drying and evaporating,

the product IIIa was crystallized once from benzene, yielding 16 g (67 %); analytical and physical data are given in Table 1.

α-Amino-oxyphenylacetic acid (IVa, Table 2). α-(N-Carbethoxyamino-oxy)carboxylic acid (IIIa, Table 1), (12 g, 0.05 mole) in 200 ml of 6 N HCl was refluxed for 1 h; the resulting solution was evaporated to dryness *in vacuo* and the residue thoroughly washed with ether, yielding α-amino-oxyphenylacetic acid hydrochloride (9 g, 88 %), m.p. 178–180°. 2 g of the hydrochloride was dissolved in 30 ml of water and the pH adjusted to 2.5–3 when IVa (1.5 g) precipitated, m.p. 158–160°. (Found: C 57.1; H 5.31; N 8.28; O 28.8. Calc. for $C_8H_9NO_3$: C 57.4; H 5.39; N 8.38; O 28.7).

Likewise were prepared from IIIb, c, d, and e (Table 1) the corresponding α-amino-oxycarboxylic acid hydrochlorides IVb, c, d, and e. Yields and melting points are given in Table 2.

α-(2-Chlorobenzylideneimino-oxy)phenylacetic acid (Vd, Table 3). α-Amino-oxyphenylacetic acid hydrochloride (2.04 g, 0.01 mole) and 2-chlorobenzaldehyde (1.55 g, 0.011 mole) were dissolved in 50 ml of ethanol. The pH of the solution was adjusted to 10–12 with 2 N NaOH. After standing for 5 h at room temperature, 200 ml of water was added and the excess of aldehyde thoroughly removed by extraction with ether. The aqueous phase was acidified with 2 N HCl, extracted with ether, the extract dried and evaporated and the residue crystallized from ligroin, yielding the Schiff base Vd (2.3 g, 80 %). Analytical and physical data are given in Table 3.

In the same manner, the Schiff bases listed in Table 3 were prepared from the α-amino-oxycarboxylic acids IVa–IVe (Table 2).

α-(N-Benzyloxycarbonylamino-oxy)phenylacetic acid (VI). α-Amino-oxyphenylacetic acid (IVa), (50 g, 0.3 mole) was dissolved at 0°C in 75 ml of 4 N NaOH. Benzyloxycarbonyl chloride (57 g, 0.33 mole) was added dropwise to the stirred solution, the pH being maintained at 9 by the simultaneous addition of 4 N NaOH. Stirring was continued for 30 min, the mixture diluted with 500 ml of water, washed with ether, the aqueous phase acidified with 2 N HCl and extracted with ether. The dried ether extract was evaporated and the oily residue crystallized twice from benzene, yielding 67 g (74 %) of product, m.p. 132–133°. (Found: C 63.91; H 5.11; N 4.46; O 26.5. Calc. for $C_{16}H_{15}NO_5$: C 63.75; H 4.98; N 4.65; O 26.6).

Resolution of α-(2-Chlorobenzylideneimino-oxy) phenylacetic acid (Vd, Table 3). To 35 g of α-(2-chlorobenzylideneimino-oxy)phenylacetic acid (Vd) in 150 ml of dry ether was added (–)-ephedrine (22 g) in 150 ml of dry ether. The precipitated salt was filtered and crystallized six times from acetone containing a little methanol until the rotation of the acid isolated from the salt as described below remained constant. Yield of pure acid, 1.5 g (9 %), $[\alpha]_D^{20}$ –95.5° (ethanol), m.p. 131–133°. The ephedrine salt was decomposed with 2 N HCl, the acid extracted with ether, the extract washed several times with 2 N HCl and saturated NaCl-solution, and the acid isolated as usual.

The mother liquors from the first two crystallizations were evaporated to dryness and from the residual salt 22.3 g of acid were isolated. This was dissolved in 150 ml of dry ether to which were added 10.4 g of (+)-benzylisopropylamine in 150 ml of dry ether. The precipitated salt was recrystallized four times from methanol until its free acid had a constant rotation; yield of pure acid, 3.5 g (20 %), $[\alpha]_D^{20}$ + 96° (ethanol), m.p. 131–133°.

Resolution of α-(N-benzyloxycarbonylamino-oxy)phenylacetic acid (VI). To VI (30.1 g) in 150 ml of dry ether was added (+)-benzylisopropylamine (13.5 g) in 100 ml of ether. The salt was isolated and recrystallized from methanol until the acid VI showed constant rotation; yield of pure acid, 7.3 g (48 %). $[\alpha]_D^{20}$ –67.7° (ethanol), m.p. 116–117°.

(–)-α-Amino-oxyphenylacetic acid hydrochloride from (–)-α-(N-benzyloxycarbonylamino-oxy)phenylacetic acid (VI). (–)-α-(N-Benzyloxycarbonylamino-oxy)phenylacetic acid (4.0 g) was heated in 6 N HCl (50 ml) for 45 min and the (–)-α-amino-oxyphenylacetic acid hydrochloride (2.0 g, 74 %) was isolated as previously described for racemic IV; $[\alpha]_D^{20}$ –85.5° (water), m.p. 143–145°.

D-(–)-Mandelic acid from (–)-α-(N-benzyloxycarbonylamino-oxy)phenylacetic acid (VI). (–)-α-(N-Benzyloxycarbonylamino-oxy)phenylacetic acid, (1 g, 0.0033 mole) was hydrogenated for 2 h over 10 % Pd on charcoal (*ca.* 20 mg) in 50 ml of ethanol in a Parr apparatus at 55 lb/in². After filtration and evaporation, the residue was dissolved in 10 ml of 2 N HCl and the acid extracted with ether. The dried ether solution was evaporated, leaving a crystalline residue; $[\alpha]_D^{20}$ –147° (acetone), m.p. and mixed m.p. with

authentic D(−)-mandelic acid = 132° (literature: $[\alpha]_D^{20}$ −147°,[19] m.p. 133.5° [20]). The IR-spectrum was identical with that of authentic D(−)-mandelic acid.

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