dl-2-Dimethylaminooxy-1-propanol Methiodide (19). Compound 14 (23.1 g, 0.253 mol) was treated with 213 g (1.5 mol) of methyl iodide and 50.7 g (0.6 mol) of $NaHCO_3$ in 210 ml of ethanol as was described for 17. The crude product was recrystallized from acetone to yield 10 g (16%) of crystals, mp 114–115°. *Anal.* ($C_6H_{16}INO_2$) C, H, I, N.

dl-2-Dimethylaminooxy-1-propyl Acetate Methiodide (8). This was prepared from 2.5 g (0.0095 mol) of 19 and 25 ml of acetic anhydride as was described for 7. The product was recrystallized from acetone: mp 124–125°; yield, 2.6 g (90%). *Anal.* ($C_8H_{18}INO_3$) C, H, I, N.

dl-2-Dimethylaminooxy-1-propyl Carbamate Methiodide (11). This was prepared from 2.6 g (0.01 mol) of 19, 1.44 g (0.02 mol) of sodium cyanate, 2.28 g (0.02 mol) of trifluoroacetic acid, and 60 ml of methylene chloride as was described for 10. The product was recrystallized from ethanol–ether to yield 1.7 g (56%) of crystals, mp 145–146°. *Anal.* ($C_7H_{17}IN_2O_3$) C, H, I, N.

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References

(1) L. L. Darko, J. G. Cannon, J. P. Long, and T. F. Burks, *J. Med. Chem.*, 8, 841 (1965).
(2) B. J. R. Nicolaus, G. Pagani, and E. Testa, *Helv. Chim. Acta*, 45, 358 (1962).
(3) P. Schiatti and G. Maffii, *Boll. Soc. Ital. Biol. Sper.*, 38, 1823 (1962).
(4) F. Winternitz and R. Lachazette, *Bull. Soc. Chim. Fr.*, 644 (1958).
(5) G. B. Bachman and T. Hokama, *J. Amer. Chem. Soc.*, 81, 4223 (1959).
(6) J. G. Cannon, T. L. Gruen, A. B. Rege, and J. P. Long, *J. Med. Chem.*, 15, 71 (1972).
(7) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, 96, 99 (1949).
(8) C. L. Butler, A. G. Renfrew, and M. Clapp, *J. Amer. Chem. Soc.*, 60, 1472 (1938).
(9) A. Hantsch, *Ber.*, 27, 1254 (1894); A. T. Fuller and H. King, *J. Chem. Soc.*, 963 (1947).

Improved Synthesis of DL-Alanosine†

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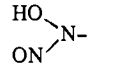
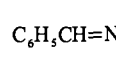
The antibiotic and antitumor agent alanosine was originally isolated as a levorotatory substance from the fermentation broth of *Streptomyces alanosinicus*.[1,2] The compound was subsequently found to have immunosuppressive activity.[3] The structure of alanosine was established as L-(−)-2-amino-3-(hydroxynitrosamino)propionic acid (L-I) by Lancini, *et al.*,[4] who described a synthesis used to prepare the D, L, and DL forms of the compound. Their approach was based upon the exothermic conversion of the β-chloroalanine derivative DL-II to DL-2-amino-3-(hydroxyamino)-propionic acid (DL-III) by means of hydroxylamine in the absence of solvent. Resolution of *N,N'*-dibenzoyl-DL-III by way of its cinchonine salt gave D-III and L-III upon removal of the blocking groups. This was followed by nitrosation using $NaNO_2$ and cold aqueous HCl to give D-I, L-I, and DL-I, L-I being identical in every respect with alanosine as obtained by fermentation (see Table I).

We now wish to report an improved chemical synthesis of DL-alanosine in which the key intermediate DL-III was prepared in a convenient alternative way through the nitrone DL-IV and in which the final nitrosation step was revised so as to facilitate the preparation of pure DL-alanosine in a stable condition.

Table I

Compd	$XCH_2CHCOOY$ (NHZ) X	Y	Z
I		H	H
II	Cl	CH_3	$COCH_3$
III	HONH–	H	H
IV		C_2H_5	$COCH_3$
V	Cl	CH_3	H·HCl
VI	Cl	CH_3	$COOC_2H_5$

Compound DL-IV, obtained from the reaction of DL-II with sodium *anti*-benzaldoximate in absolute ethanol, was hydrolyzed with concentrated HCl to the desired amino acid DL-III. The intermediate DL-IV need not be isolated in this synthesis, it being more efficient to proceed directly from DL-II to DL-III (yield, 66%). In a separate experiment the nitrone DL-IV was isolated (yield, 7%) and characterized as an ethyl ester, indicating that ester interchange had taken place with the ethanol solvent.

This revised approach avoids the potentially hazardous reaction of DL-II with the free base hydroxylamine, which is itself difficult to prepare. Furthermore, we have found our method to be readily adaptable to the preparation of large quantities (*ca.* 100 g) of DL-III. Several attempts to convert DL-II into DL-III using solutions of hydroxylamine in which the base was generated from its hydrochloride gave gummy products which were not identified.

Precedent for the present approach to the introduction of the hydroxylamino group may be found in the publications of Buehler and Brown,[5] Bellasio, *et al.*,[6] and Schoenewaldt, *et al.*[7]

In studies of the synthesis of *N*-hydroxylamino acids Liberek and Palacz[8] noted that only minor racemization was encountered when an optically active α-bromocarboxylic acid was converted to a nitrone using sodium *anti*-benzaldoximate, the predominant stereochemical result being inversion. In this connection it was noted in our experiments that complete racemization occurred when the present method was applied to L-II or L-VI. Our observation that the nitrone reaction mixtures were devoid of optical activity is consistent with the report of Lancini, *et al.*,[4] that L-II gave an optically inactive product after reaction with hydroxylamine.

The final nitrosation step was revised in order to provide a product in which the levels of contamination by residual acetic acid and metallic salts were substantially lowered. This was accomplished by using an absolute minimum of NaOH solution to dissolve the product and by effecting precipitation by adding acetic acid to pH 5.4, instead of 4.0 as previously specified.[4] Product prepared according to the revised procedure was found by nmr to be free of residual acetic acid, to have an undetectable ash content, and to be stable indefinitely at 5°, in contrast to samples purified using the original procedure.

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Experimental Section

Melting points were obtained on a Thomas-Hoover Unimelt using open capillary tubes and are uncorrected. Evaporations were performed at diminished pressure on a rotary evaporator. Petroleum ether refers to that fraction boiling from 30–60°. Analyses indicated only by the symbols of the elements were within ±0.3% of the theoretical values. The ir spectra were obtained on a Perkin-Elmer Model 137 recording spectrophotometer and the uv spectra by A. Kalowsky using a Cary Model 11 spectrophotometer. A Varian Associates A-60A instrument was used by Dr. A. W. Douglas and staff for recording nmr spectra. The authors are grateful to Mr. R. N. Boos and associates for microanalyses and to Mr. T. E. Lanza for helpful advice.

DL-*N*-Acetyl-3-chloroalanine Methyl Ester (DL-II). The method of Rothstein[9] was modified so as to provide a higher yield. A suspension of 257 g (1.48 mol) of DL-3-chloroalanine methyl ester hydrochloride (DL-V, Cyclo Chemical) in 1.8 l. of anhydrous benzene was mixed with 234 g (3.0 mol) of acetyl chloride and heated under reflux for 2.5 hr. A small amount of solid was removed by filtration and into the stirred filtrate was poured 4.5 l. of petroleum ether over a 30-min period. Overnight cooling gave a solid which was collected, washed with 500 ml of petroleum ether, and dissolved in 6.5 l. of ether. The solution was evaporated to a volume of 400 ml and diluted with 1.5 l. of petroleum ether. Filtration, followed by washing with petroleum ether, gave 224 g (88%) of pure product, mp 77.5–79.5° (lit.[9] mp 79–80°).

DL-2-Amino-3-(hydroxyamino)propionic Acid (DL-III). Under anhydrous conditions 77.9 g (0.43 mol) of DL-II was dissolved in 710 ml of absolute ethanol and to the solution was added over 1.2 hr a solution of sodium *anti*-benzaldoximate prepared by adding 105 g (0.87 mol) of *anti*-benzaldoxime[7] to a solution of 19.9 g (0.87 g atom) of sodium metal in 710 ml of absolute ethanol. The reaction mixture was neutralized with 30 ml of saturated ethanolic HCl, cooled to 4°, filtered, and evaporated to give 187 g of an oil. This was heated under reflux with 850 ml of concentrated HCl for 30 min. Most of the HCl gas was next removed by evaporation in a bath at 45°, causing a solid to separate which was removed by filtration. The filtrate was washed with three 850-ml portions of ether and concentrated to dryness. The residue was evaporated with three small portions (*ca.* 50 ml) of H_2O and finally taken up in 180 ml of H_2O. This solution was washed with six 400-ml portions of ether, separated, and cooled to 3°, and the pH was adjusted to 6.5 by the addition of concentrated aqueous ammonia. The solution was treated with Darco KB, filtered, and cooled to 0°, and to it was added 2.23 l. of absolute ethanol with stirring over 1.2 hr. The solid that separated was collected and washed with ethanol and ether to yield 34.4 g (66%) of the product, mp 180–181° dec (lit.[4] mp 163–165° dec). The compound reduced Fehling's solution and its ir spectrum (Nujol) was superimposable upon that of an authentic sample.‡

DL-2-Amino-3-(hydroxynitrosamino)propionic Acid (DL-I). A solution of 91.1 g (0.76 mol) of DL-III in 765 ml of 1 *N* HCl was treated (17 min) at 0° with 52.3 g (0.76 mol) of $NaNO_2$. A solid began to separate after about half of the nitrite had been added. Addition of 1.1 l. of cold absolute ethanol completed the precipitation of the product, which was filtered and washed with ethanol and ether to give 97.5 g (86%) of slightly yellow powder (air dried), mp 189° dec. Purification was performed by dissolving 75.0 g (0.504 equiv) in 475 ml of 1 *N* NaOH (0.475 equiv) to give a faintly turbid solution (pH 6.2). This was treated with Darco KB, filtered, and cooled in an ice bath, and the pH was adjusted to 5.4 by the dropwise addition of 28 ml of acetic acid. The precipitate was collected and washed successively with 100-ml portions of H_2O, ethanol, and ether. Drying over KOH pellets at diminished pressure afforded 60.3 g (79%) of ash-free, white powder, mp 193° dec (lit.[4] mp 185°). Uv, ir, and nmr spectra of the product were in accord with expectation.

DL-*N*-(2-Acetamido-2-carboxyethyl)-α-phenylnitrone Ethyl Ester (DL-IV). A solution of 0.01 mol of sodium *anti*-benzaldoximate in 40 ml of absolute ethanol, prepared as prescribed above, was combined with 1.8 g (0.01 mol) of L-*N*-acetyl-3-chloroalanine methyl ester (L-II), $[\alpha]^{25}D$ −16.4° (*c* 0.93, H_2O), prepared as described in the literature,[10] and heated at 65–70° for 45 min. Concentration to dryness gave 3.0 g of an oil which solidified when treated with 15 ml of ether. The optically inactive solid (1.0 g) was dissolved in absolute ethanol (3 ml), evaporated to dryness, and triturated with ether to yield 200 mg (7%) of product: mp 128–130°; $\lambda_{max}^{ethanol}$ [nm ($\epsilon \times 10^{-3}$)] 296 (20.0), 229 (7.3), and 224 (8.3); ir and nmr spectra were as expected. *Anal.* ($C_{14}H_{18}N_2O_4$) C, H, N.

DL-*N*-Carbethoxy-3-chloroalanine Methyl Ester (DL-VI). Under anhydrous conditions 5.2 g (0.03 mol) of DL-V was mixed with 7.5 g (0.09 mol) of $NaHCO_3$ and 6.5 g (0.06 mol) of ethyl chloroformate. The mixture was stirred for 4.5 hr at room temperature and filtered and the filtrate was concentrated to give 5.3 g (84%) of product, mp 50–56°. The analytical sample was crystallized from petroleum ether–ether: mp 64–66°; ir and nmr spectra were as expected; tlc, single spot, R_f 0.9, silica gel G, ethyl acetate, visualized with I_2 vapor. *Anal.* ($C_7H_{12}ClNO_4$) C, H, Cl, N.

L-*N*-Carbethoxy-3-chloroalanine Methyl Ester (L-VI). This stereoisomer was prepared in the manner described above for the DL form, starting with L-V (Cyclo Chemical). The product was obtained as crystals from petroleum ether–ether in 75% yield; mp 57–59°; $[\alpha]^{25}D$ −29° (*c* 2, dimethylformamide). Attempts to react L-VI with sodium *anti*-benzaldoximate under various conditions resulted in complete loss of optical activity in the reaction products, even under mild conditions.

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References

(1) Y. K. S. Murthy, J. E. Thiemann, C. Coronelli, and P. Sensi, *Nature (London)*, 211, 1198 (1966).
(2) C. Coronelli, C. R. Pasqualucci, G. Tamoni, and G. G. Gallo, *Farmaco, Ed. Sci.*, 21, 269 (1966).
(3) D. Fumarola, *Pharmacology*, 3, 215 (1970).
(4) G. C. Lancini, E. Lazzari, and A. Diena, *Farmaco, Ed. Sci.*, 24, 169 (1969).
(5) E. Buehler and G. B. Brown, *J. Org. Chem.*, 32, 265 (1967).
(6) E. Bellasio, F. Parravicini, A. Vigevani, and E. Testa, *Gazz. Chim. Ital.*, 98, 1014 (1968).
(7) E. F. Schoenewaldt, R. B. Kinnel, and P. Davis, *J. Org. Chem.*, 33, 4270 (1968).
(8) B. Liberek and Z. Palacz, *Rocz. Chem.*, 45, 1173 (1971).
(9) E. Rothstein, *J. Chem. Soc.*, 1968 (1949).
(10) L. Benoiton, *Can. J. Chem.*, 46, 1549 (1968).

The N^6-(Dimethylamino)methylene Derivative of 9-β-D-Arabinofuranosyladenine as an Antiviral Agent

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Recent reports[1,2] from these laboratories presented information on the antiviral compound, 9-β-D-arabinofuranosyladenine (Ara-A), which shows significant therapeutic activity against herpes simplex and vaccinia viruses in cell cultures and experimental animals. In addition, Ara-A exhibits an inhibitory effect on a variety of other DNA viruses in cell culture but little if any on RNA viruses.[1,3]

The antiviral activity of nucleosides may be limited or altered due to enzymatic or chemical transformation *in vivo*. The distribution and metabolic fate of such compound may thus be affected by such processes. In this regard it is noteworthy that unlike its 2 epimer adenosine, Ara-A is relatively insoluble in water (≃1 mg/ml). It seemed desirable to prepare derivatives of Ara-A that would combine the properties of greater solubility in water and the regeneration of the parent nucleoside in a sustained fashion.

We wish to report in this note on the preparation of a water-soluble derivative of Ara-A and to comment on its antiviral activity and related properties. The new derivative is the N^6-(dimethylamino)methylene analog **1** (Scheme I) which was prepared in high yield by the reaction of Ara-A with *N,N*-dimethylformamide dimethyl acetal[4] in DMF.

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