promoting the present reaction will be made the subject of further study.

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The Synthesis of Hydroxylysine from the Cyanohydrin of γ-Acetamido-γ,γ-dicarbethoxybutyraldehyde[1]

BY OSCAR TOUSTER

Bergström and Lindstedt[2] and Sheehan and Bolhofer[3] have recently presented degradative evidence which establishes the structure of natural hydroxylysine as α,ε-diamino-δ-hydroxycaproic acid. The latter workers also synthesized this amino acid by two routes.[4] The first method was based upon the reduction and hydrolysis of methyl α,ε-diphthalimido-δ-keto-DL-caproate, which was originally prepared in several steps from glutamic acid.[3] The second synthesis involved the catalytic hydrogenation of the cyanohydrin of γ-acetamido-γ,γ-dicarbethoxybutyraldehyde[5] in acetic anhydride, followed by hydrolysis and decarboxylation of the reduction product. We have independently synthesized hydroxylysine from this cyanohydrin by a similar procedure, except that crystalline cyanohydrin was employed in our work and the amino acid was isolated in a different manner. The yield of crystalline hydroxylysine was 40%, based on the cyanohydrin. This initial product and material obtained from it by recrystallization in 60% yield gave similar amounts of formaldehyde on reaction with periodate. The identity of the amino acid was established by elementary analyses of its monohydrochloride, monopicrate and dipicrate, by its reaction with periodate to form formaldehyde and ammonia, by its behavior in a new modified ninhydrin test and by paper chromatography.

Experimental

Nine grams of the cyanohydrin of γ-acetamido-γ,γ-dicarbethoxybutyraldehyde[6] was dissolved in 135 ml. of a 3:1 mixture of ethyl acetate and acetic anhydride and hydrogenated for 6 hours at three atmospheres pressure and room temperature, using 0.6 g. of Adams platinum oxide as catalyst. After removal of the catalyst by filtration and removal of solvents by distillation *in vacuo*, the colorless product was dissolved in 70 ml. of concd. HCl and the solution refluxed for 15 hours. The acid was then removed under reduced pressure and an aqueous solution of the residue decolorized with charcoal. Evaporation to dryness yielded 7.2 g. of crude dihydrochloride as a yellow oil which partially crystallized on drying over phosphorus pentoxide.

Three and five-tenths grams of the crude dihydrochloride was dissolved in 60 ml. of hot 95% ethanol and treated with 1.63 ml. of aniline in 5 ml. of 95% ethanol, followed by sufficient water (10 ml.) to prevent oiling out of monohydrochloride. The solution was allowed to stand at room temperature for a short time and then overnight in the refrigerator. The first crop of hydroxylysine monohydrochloride (0.61 g.) was collected by filtration and washed with ethanol. Cooling of the combined mother liquor and washings yielded a considerable amount (0.56 g.) of additional monohydrochloride. The total yield of amino acid, based on cyanohydrin, was 40%. A slightly smaller yield was obtained when pyridine was employed in place of aniline. Recrystallization from twenty volumes of 70% ethanol gave a product which decomposed at 215–220°.[7]

Anal.[8] Calcd. for $C_6H_{15}N_2O_3Cl$: C, 36.27; H, 7.61; N, 14.10. Found: C, 36.65; H, 7.58; N, 14.62; NH_2-N, 14.3.

An orange-yellow monopicrate was prepared by reaction of hydroxylysine with one equivalent of picric acid in aqueous solution. A small amount of light yellow dipicrate also formed. This was separated manually and the monopicrate was then recrystallized from water. It melted at 224–226°.

Anal. Calcd. for $C_{12}H_{17}N_5O_{10}$: C, 36.83; H, 4.38; N, 17.90. Found: C, 37.26; H, 4.25; N, 17.62.

The dipicrate was obtained by using slightly more than two equivalents of picric acid. It melted at 123–125°[9] after recrystallization from water.

Anal. Calcd. for $C_{18}H_{20}N_8O_{17}$: C, 34.85; H, 3.25; N, 18.06. Found: C, 34.97; H, 3.47; N, 17.76.

Synthetic and natural hydroxylysine monohydrochloride showed identical behavior in a new modified ninhydrin test which distinguishes hydroxylysine from other natural amino acids.[10] Using a periodate oxidation procedure in which natural hydroxylysine has been reported to yield ammonia and a 65% yield of formaldehyde as the dimedone condensation product,[11] ammonia and a 59% yield of formaldehyde–dimedone product were obtained from the synthetic preparation. The synthetic and natural amino acids had identical R_f values on paper chromatograms run in phenol–hydrochloric acid and phenol–ammonia systems.[12]

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[1] Supported in part by The Rockefeller Foundation and Nutrition Foundation, Inc., through grants to the Division of Nutrition.

[2] Bergström and Lindstedt, *Arch. Biochem.*, **26**, 323 (1950).

[3] Sheehan and Bolhofer, THIS JOURNAL, **72**, 2469 (1950).

[4] Sheehan and Bolhofer, *ibid.*, **72**, 2472 (1950).

[5] Moe and Warner, *ibid.*, **70**, 3918 (1948).

[6] We are indebted to Dr. O. A. Moe, of General Mills, Inc., Minneapolis, Minn., for a sample of this compound.

[7] Melting points were taken with a Fisher–Johns microblock.

[8] Microanalyses by Clark Microanalytical Laboratory, Urbana, Illinois.

[9] The different melting point behavior observed by Sheehan and Bolhofer[4] was probably due to difference in proportion of racemates or different technique for determining melting point, or both.

[10] An unpublished test of Dr. Francis P. Chinard of Johns Hopkins University. We are indebted to Dr. James Weisiger of the Hospital of the Rockefeller Institute for Medical Research for information regarding this test and for a sample of natural hydroxylysine.

[11] A. P. J. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 309 (1941).

[12] We are indebted to Dr. J. G. Heathcote, Cereals Research Station, St. Albans, Herts, England, for the paper chromatographic comparisons.

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The Solution Absorption Spectrum of Americium

BY B. J. STOVER,* J. G. CONWAY AND B. B. CUNNINGHAM

It has long been observed that the absorption spectra of the trivalent rare earth ions possess a unique sharpness, the structure of the peaks being discernible at room temperature and even in solutions. This phenomenon has also been observed in the case of the electronically analogous actinide elements.

The generally accepted explanation for the origin of the lines in the rare earth spectra is that they result from transitions within the 4f electron shell.

Trivalent europium, the analog of americium, has been shown by absorption spectra and magnetic susceptibility data to have a $4f^6$ electron configuration, a $7F_0$ ground state, and one or more low lying excited levels. Since americium ion probably has a $5f^6$ configuration, it also is expected to have a singlet ground state and one or more low lying excited levels. The following represents the first

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phases of the study of the absorption spectrum of americium.

A Baird Eagle Mount Spectrograph, having 5.6 Å./mm. dispersion in the first order, was used for the solution work. A 1 cm. path length cell 2 mm. wide was mounted on the arm of a micro-manipulator. The cell was then placed as close to the slit as possible. The cell was of fused silica. For the wave lengths below 3500 Å. a water cooled hydrogen discharge lamp was used and above 3500 Å. a tungsten filament lamp.

The absorption spectrum of trivalent americium in perchloric acid solution has been measured in the wave length range 2200 to 10,000 Å., and the results appear in Table I. The spectrograms show a marked similarity to those of rare earth solutions, but the americium absorption is much more intense. The absorption in the 5000 Å. region is especially strong and was found to persist at very low concentrations.

TABLE I

Am^{+++} IN $HClO_4$ AT ROOM TEMPERATURE

λ, Å.	8133	5166	5104	5078	5030	4543	4287
λ, Å.	4198	4018	3784	3771	3756	3605	3391
λ, Å.	3353	3312	2893	2861	2847	2826	2754
λ, Å.	2705	2699	2651	2597	2537	2436	2433

The measurements were made using a solution 0.04 *M* in americium and 0.5 *M* in perchloric acid. However, to detect the four components of the 5000 Å. peak, it was necessary to reduce the americium concentration to about 10% of the above. With this solution none of the other lines were discernible.

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Some Synthetic Esters of Germine

BY HOWARD L. WHITE

The hypotensive principles of *Veratrum viride*, germidine and germitrine, have been characterized as mixed di- and triesters, respectively, of the alkamine germine.[1] The constituting acids in germidine are acetic acid and *l*-α-methylbutyric acid, while germitrine contains in addition *d*-methylethylglycolic acid. Removal of the acyl groups by hydrolysis all but abolishes the hypotensive activity. Hence it was of interest to prepare simple synthetic esters of germine and to compare their hypotensive potency with that of the natural ester alkaloids.

Germine, $C_{27}H_{43}O_8N$, is an octahydric alcohol in which five hydroxyl groups are esterifiable.[2] Esterification with acyl chlorides in cold pyridine solution should therefore lead primarily to pentaesters. However, the crude products thus obtained invariably proved to be complex mixtures, much to the detriment of the yield of crystalline esters isolated. In the majority of cases no crystalline products were realizable even when resort was taken to chromatographic fractionation. The properties of the four new crystalline esters obtained are listed in Table I, together with those of germine and of germine pentaacetate.[1] As can be seen, acylation of all five reactive hydroxyl groups was attained in only two cases. It is evident that although esterification greatly potentiates the (weak) hypotensive effect of germine, the potency of the best compound of the series, the triisobutyrate, still falls short of that of the natural ester alkaloid, germitrine, by a factor of about 1:12.[3]

TABLE I

	M. p. °C.	$[\alpha]_D$, in pyridine	Minimum effective dose[a] µg./kg.
Germine	208 (dec.)	− 2.5	2000
pentaacetate	256–257	−85	300–400
tetrapropionate	216–217	−92.4	300–400
triisobutyrate	197–201	−65.8	6–8
pentacyclohexane-carboxylate	201–205	−60.4	32
tetrabenzoate	223–225	−68.1	300–400
Germitrine	216–219	−69	0.5

[a] By intravenous route in the anesthetized dog. The assays involved comparison of the hypotensive effect with that given by a standard preparation ("total amorphous bases"[1]) having one-fourth of the hypotensive potency of germitrine.

All the synthetic esters showed high specific rotation in pyridine, of the order of that of germitrine. In the natural triester this property is specifically associated with its acetoxy group, since it is no longer in evidence in the de-acetylated diester, germerine, formed from germitrine by methanolysis.[1] Hence it appears probable that the hydroxyl group which carries the acetyl group in germitrine is also esterified in all the synthetic esters.

Esterification of germine with *n*-valeric, isovaleric, diethylacetic, piperonylic and veratric acids failed to yield crystalline products even after chromatographic fractionation. The hypotensive potency of the main fractions, while invariably exceeding that of germine, was generally of too low an order to warrant further investigation.

Experimental

Germine Tetrabenzoate.—A solution prepared by adding at 0° freshly distilled benzoyl chloride (564 mg., 4.0 millimoles) in dry pyridine (4 cc.) to germine (250 mg., 0.5 millimole) dissolved in the same solvent (4 cc.) was allowed to stand at 0° for 19 hours. It was then worked up by pouring it into a saturated sodium bicarbonate solution containing excess chopped ice, and extracting after 30 minutes with four 50-cc. portions of chloroform. The brown oil (450 mg.) obtained by evaporation *in vacuo* of the dried extract was triturated for a short time with a 1:1 mixture of 5% acetic acid and 10% sodium chloride solution. The resulting microcrystalline hydrochloride was filtered off (m.p. of a dried sample 187–188°), decomposed with saturated sodium bicarbonate solution, and the basic ester recovered by chloroform extraction. The product crystallized in part when its solution in absolute ethanol was allowed to stand at 4°. After three recrystallizations from the same solvent the pure ester (50 mg.) melted at 223–

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[1] J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **72**, 4621 (1950).

[2] W. Poethke, (a) *Arch. Pharm.*, **275**, 357 (1937); (b) *ibid.*, **275**, 571 (1937); (c) *ibid.*, **276**, 170 (1938).

[3] A preliminary clinical assay conducted on three hypertensive patients by Dr. E. D. Freis of the Veterans' Administration Hospital, Washington, D. C., showed the potency of the triisobutyrate to be about one-fortieth that of germitrine by the intravenous route.