alkyl halide[29] (0.043 mole) were heated at 100–110° in a pressure bottle for 20 hours. A solution of 2.4 g. (0.043 mole) of potassium hydroxide in 10 cc. of water was added to the cooled, stirred mixture. The product was filtered and washed successively with water, ethanol and ether.

In the preparation of compound 7, 50% ethanol was used as a solvent. To isolate the product, the reaction mixture, after addition of the potassium hydroxide solution, was extracted with chloroform, the extract was evaporated to dryness and the residue was washed with ether.

To prepare compound 9 (XVI), the reaction mixture was merely heated on a steam-bath for 5 minutes.

In the preparation of compound 13, twice the usual quantity of potassium hydroxide was used, and the basic halide was added as the hydrochloride. After heating for 1 hour, the precipitated basic thio ether was filtered and washed, successively, with isopropyl alcohol and ether; yield 3.7 g., m.p. 182–183°. A filtered chloroform solution was treated with hydrogen chloride, the solvent was removed and the oily residue was solidified by rubbing it with ether and then with ethanol.

When the preparation of compound 8 was carried out by the general procedure, a mixture (8.4 g.) of compound 8 and 1,3,9-trimethyl-7-benzyl-8-thiouric acid (XV) was obtained. In order to separate the two isomers, the mixture was extracted with concentrated hydrochloric acid and the insoluble solid (A) was treated as described below. The acidic extract was diluted with water and then neutralized with potassium carbonate to precipitate compound 8.

To obtain XV, the insoluble material (A) was washed with ethanol and ether (yield 1.1 g., 10%) and recrystallized from 2-butanone; m.p. 190–191°.

Anal. Calcd. for $C_{15}H_{16}O_2N_4S$: C, 56.94; H, 5.10: N, 17.71; S, 10.13. Found: C, 56.80; H, 5.08; N, 17.92; S, 10.27.

A mixed melting point of compound 8 with XV was found to be 165–190°. A qualitative test for a thio ether linkage[12] was positive for compound 8 but negative for XV.

Compound 11 was obtained when 6.0 g. of compound 9 and 50 cc. of acetic anhydride were heated for 4 hours on a steam-bath. The excess anhydride was removed *in vacuo*, the residue was washed successively with water, ethanol and ether, and the material was then extracted with chloroform. The extract was evaporated to dryness and the residue was recrystallized from 2-butanone. When the preparation of 11 was attempted by the general procedure described above, compound 9 was obtained.

(29) The iodide was used for the preparation of compound 1, the bromide for compounds 2–10 and the chloride for compounds 12 and 13. Compound 11 was prepared by acetylation of 9.

Four grams of compound XII, 3.0 g. of chloroacetic acid and 10 cc. of water were refluxed for 48 hours, and the precipitate was washed successively with water, ethanol and ether. The product was the 8-carboxymethyl derivative (compound 12).[30]

1,3,9-Trimethylisoxanthine (XIV).—One gram of 1,3,9-trimethyl-8-methylthioisoxanthine (XIII, R = CH_3), 10 cc. of water and 3.3 g. of Raney nickel[21] were refluxed for 1 hour. The hot mixture, which smelled strongly of methyl mercaptan, was filtered, the nickel was washed with hot water, and the filtrate and wash water were combined and evaporated to dryness. The residue was extracted with chloroform and the insoluble material was recrystallized twice from dilute ethanol; yield 0.2 g. (25%), m.p. 285–286°. A mixed melting point with XIV (m.p. 285–287°), which had been prepared from III (R = CH_3), was 285–287°.

Decomposition of XVI. (A).—Ten grams of XVI was heated at 240–260° in a nitrogen stream for 30–60 minutes and the evolved ethylene sulfide (1 cc., micro b.p. 55°)[31] was condensed in an ice-cold receiver. The residue was extracted with chloroform and the extract was discarded. The insoluble material was dissolved in dilute potassium hydroxide solution, the solution was extracted with chloroform, and the water layer was acidified. The precipitate, 1,3,9-trimethyluric acid (XVII), was washed with ethanol and ether (yield 5.0 g., 64%) and recrystallized from water; m.p. 340° dec.[32]

Anal. Calcd. for $C_8H_{10}O_3N_4$: C, 45.71; H, 4.80; N, 26.66. Found: C, 45.96; H, 4.63; N, 26.70.

The acetyl derivative melted at 230–236°.[33]

(B).—Three grams of XVI and 150 cc. of water were refluxed for 80 hours. The hot mixture was filtered, the filtrate was cooled and made basic with dilute potassium hydroxide solution. The filtered solution was acidified with concentrated hydrochloric acid and evaporated to a small volume. The precipitate (XVII) was washed with water, ethanol and then with ether; yield 1.9 g. (81%). m.p. 340° dec.

(30) In some instances, thio compounds have been converted into corresponding hydroxy compounds by the action of chloroacetic acid (W. J. Croxall, C. Lo and E. Y. Shropshire, THIS JOURNAL, **75**, 5419 (1953)).

(31) This compound exhibited the properties described by M. Delepine (*Bull. soc. chim.*, [4] **27**, 740 (1920)).

(32) H. Biltz and H. Pardon (*Ber.*, **63**, 2876 (1930)) reported m.p. 340° dec.

(33) H. Biltz and H. Pardon (*J. prakt. Chem.*, [2] **134**, 310 (1932)), m.p. 235°.

ANN ARBOR, MICHIGAN

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

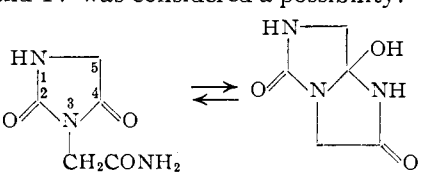
Some Reactions of 3-Substituted Hydantoins

BY LOUIS A. COHEN AND EDWARD M. FRY

RECEIVED JULY 2, 1956

A variety of chemical methods have been used to search for amine–carbonyl interaction in hydantoinacetamides (III). Such interaction was not observed. Improved methods for the cyclization of carbobenzyloxydipeptide derivatives to the corresponding hydantoins are described as well as some novel transformations of hydantoinacetonitriles.

The facile interaction of a peptide nitrogen with a ketonic carbonyl has been reported recently from this Laboratory.[1] As part of a study of peptide bond reactivity, we have investigated the possibility of NH· · ·CO interaction in hydantoin-3-acetamides (for example, III ⇄ IV). Since the reactivity of the 4-carbonyl of hydantoins may be considered intermediate between that of an amide and a ketone,[2] the existence of an equilibrium mixture of III and IV was considered a possibility.



III IV

Among the numerous methods available for the

(1) L. A. Cohen and B. Witkop, THIS JOURNAL, **77**, 6595 (1955).

(2) (a) I. J. Wilk and W. J. Close, *J. Org. Chem.*, **15**, 1020 (1950); (b) L. Crombie and K. C. Hooper, *J. Chem. Soc.*, 3010 (1955).

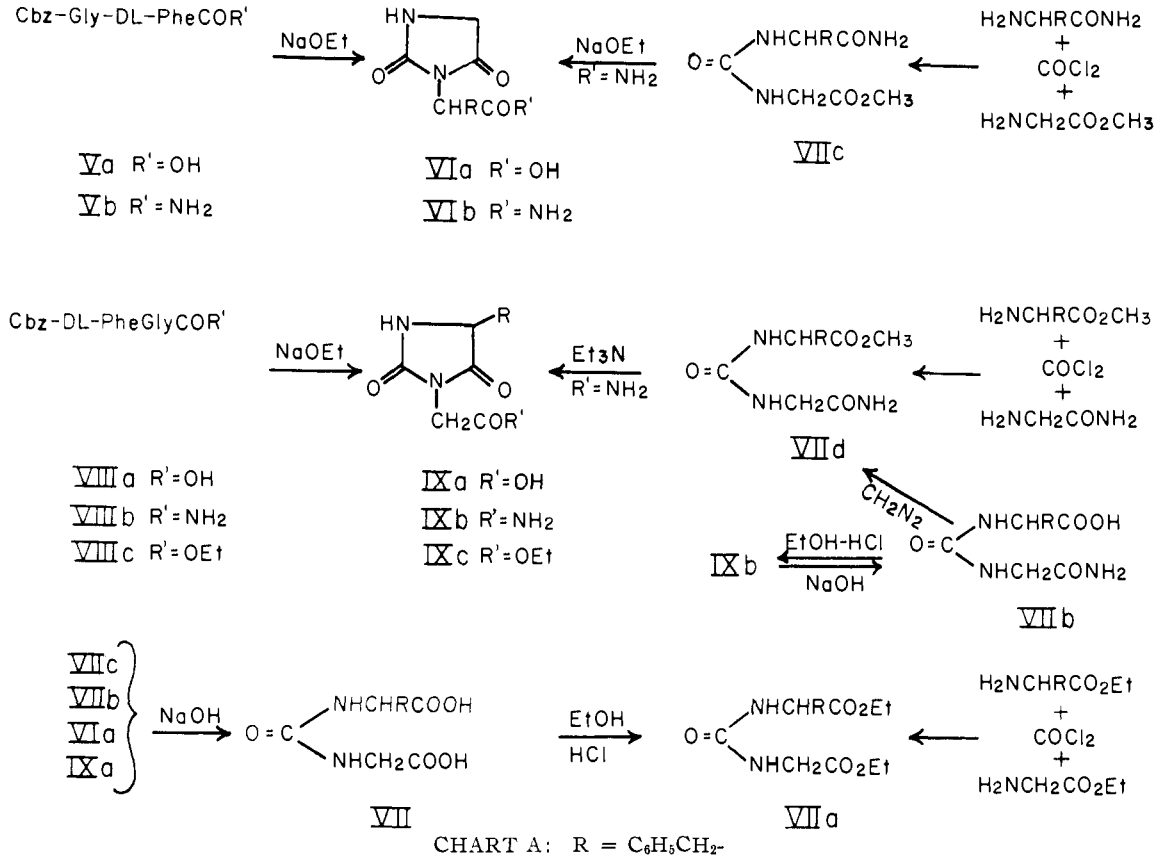
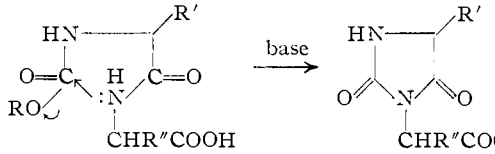
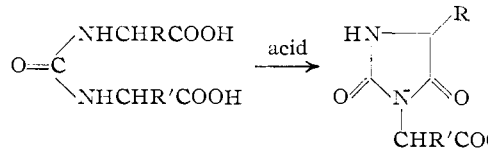


CHART A: R = $C_6H_5CH_2$-

synthesis of hydantoin-3-acetic acid and its derivatives,[3] of particular utility are the ring closure of carbalkoxypeptides with alkali[4] and the acid cyclization of carbonyl-N,N'-bisamino acids.[5,6] An extension of the former method was described by





Fruton and co-workers in the isolation of hydantoin-3-acetamides from the reaction of several carbobenzyloxydipeptide esters with ammonia.[7] By conducting the ammonolysis under somewhat milder conditions, we were able to isolate a mixture of the carbobenzyloxydipeptide amide and the hydantoinacetamide, suggesting that the former is formed prior to cyclization. Further, the use of alcoholic sodium ethoxide at room temperature provided a general method for converting carbobenzyloxydipeptide acids, esters or amides into the corresponding derivatives of hydantoin-3-acetic acid. Chart A summarizes a number of syntheses and transformations of hydantoin derivatives which are more fully described in the Experimental Section.

Despite the fact that the infrared spectra of the hydantoin-3-acetamides (VIb and IXb) showed the usual absorption at 5.61–5.65 μ characteristic of a 4-carbonyl,[8] we felt that the existence of an equilibrium mixture of the open and closed forms (III and IV) merited consideration

Three methods were used in attempting to demonstrate the existence of IV. It had previously been shown that the carbinolamine resulting from interaction of a ketone and an amide nitrogen was subject to catalytic hydrogenolysis.[1] When the method was applied to IXb, only hydrogenation of the aromatic nucleus could be realized.

Earlier studies with hydantoins[9] have demonstrated that a 5-substituted hydantoin is favored in stability over an unsubstituted one. Thus, the acid cyclization of X will form XI apparently to the complete exclusion of XII. It was postulated that, during the cyclization of Vb to VIb, the strong alkali present might induce the rearrangement de-

(3) E. Ware, *Chem. Revs.*, **46**, 403 (1950).

(4) (a) Reference 3, p. 420; (b) F. Wessely, E. Kemm and J. Mayer, *Hoppe-Seyler's Z. physiol. Chem.*, **180**, 64 (1929).

(5) Ch. Gränacher and H. Landolt, *Helv. Chim. Acta*, **10**, 799 (1927).

(6) The naming of such compounds as derivatives of the amino acids to which they are related is found extensively in the literature. It was considered desirable to maintain the trivial name rather than create a system based on urea-1,3-diacetic acid or on 1,3-dicarboxymethylurea.

(7) J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **145**, 253 (1942); C. A. Dekker, S. P. Taylor and J. S. Fruton, *ibid.*, **180**, 155 (1949).

(8) The position of the 4-carbonyl band was identical for hydantoin ester, amide or nitrile; *cf.* ref. 2b.

(9) (a) K. Schlögl, F. Wessely and G. Korger, *Monatsh.*, **83**, 502 (1952); (b) F. Wessely, K. Schlögl and E. Wawersich, *ibid.*, **83**, 1432 (1952); (c) ref. 3, p. 447.

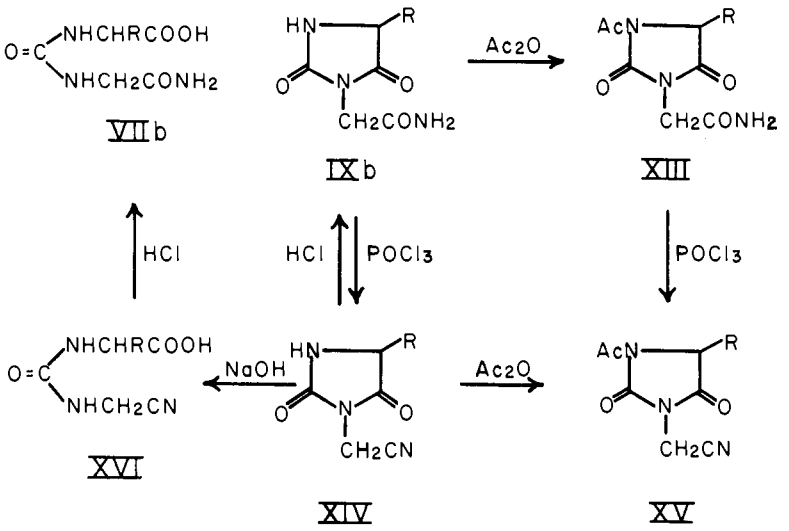


CHART B: R = $C_6H_5CH_2$-

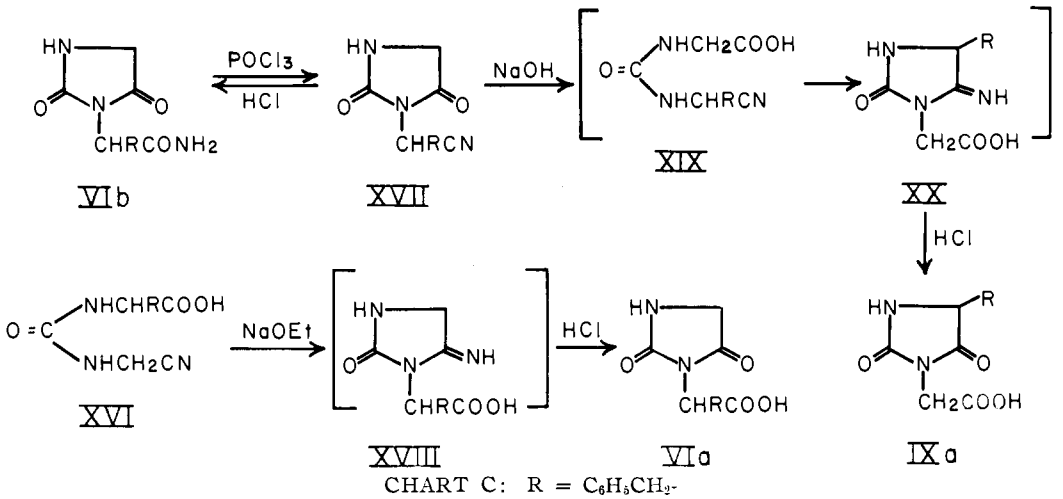
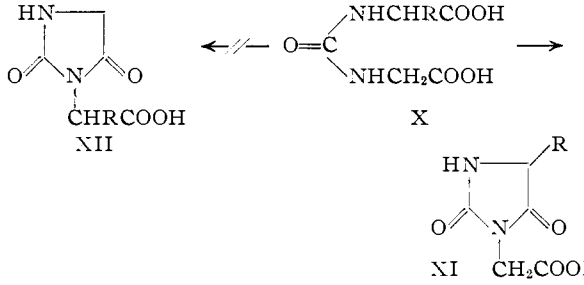
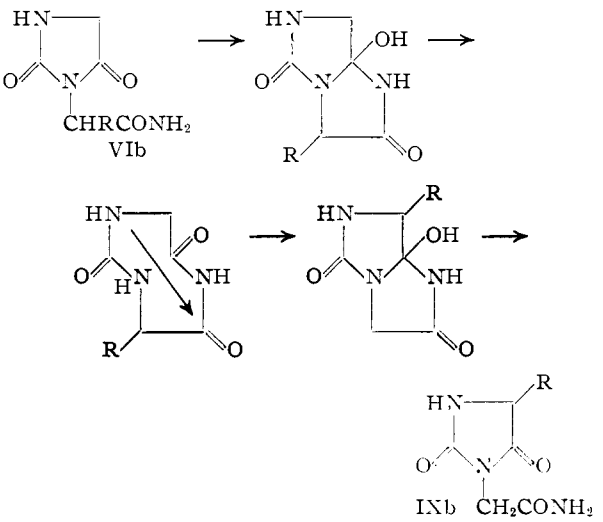


CHART C: R = $C_6H_5CH_2$-

scribed below as a means of attaining greater stability. However, VIb was the only product obtained from the cyclization.



Finally, the reactions of IXb with acetic anhydride and with phosphorus oxychloride were studied. The results are summarized in Chart B. Since XV showed no infrared absorption in the region of 3.0 μ, acetylation apparently had occurred at N-1, in agreement with earlier studies on the acetylation of hydantoins.[10] The stability of the acetyl derivative rendered its formulation as an enol acetate at C-2 unlikely. The course of the reaction with phosphorus oxychloride was not immediately



(10) (a) M. R. Salmon and A. Z. Kozlowski, THIS JOURNAL, **67**, 2270 (1945); (b) L. Siemonsen, *Ann.*, **333**, 130 (1904).

recognized because of the absence of nitrile absorption in the infrared spectrum. The lack of absorption for nitriles carrying an α-oxygen or nitrogen substituent appears to be a general phenomenon.[11]

From these experiments it may be inferred that IXb *reacts* primarily as the monocyclic amide or, more likely, that it *exists* as the open form in its ground state.

When XVI was treated with sodium ethoxide solution, the isomeric hydantoin-3-acetic acid was obtained. The over-all reaction sequence, as outlined in Chart C, results in the shift of a 5-alkyl substituent on the hydantoin ring to the α-position of the acetic acid side chain. The enhanced stability of a 5-substituted hydantoin was demonstrated in the reverse sequence where aqueous alkali sufficed to cyclize XIX to XX. In both cyclizations the sodium salt of the intermediate imino acid could be separated, but no attempt was made at characterization.

Experimental[12]

Carbobenzyloxy-DL-phenylalanylglycine Ethyl Ester (VIIIc).—To a solution of 6.0 g. (0.02 mole) of carbobenzyloxy-DL-phenylalanine in 50 ml. of methylene chloride was added 2.8 ml. (0.02 mole) of triethylamine. The solution was chilled to −5° and 1.9 ml. (0.02 mole) of ethyl chlorocarbonate was added from a pipet. The flask was kept at −5° for 10–15 min. during which time some triethylamine hydrochloride separated. In another flask 8.4 ml. (0.06 mole) of triethylamine was added to a chilled suspension of 5.6 g. (0.04 mole) of glycine ethyl ester hydrochloride in 50 ml. of chloroform. The resulting solution was added to that in the first flask and the mixture kept for 1 hr. at 25°. The solution was washed successively with water, *N* hydrochloric acid and 5% sodium bicarbonate, dried over sodium sulfate and concentrated under reduced pressure. The crystalline residue was triturated with ether and filtered. On standing, the ethereal filtrate deposited additional material; total wt. 5.7 g. (74%), m.p. 96–98°. Purified from alcohol, it melted at 97–98.5°.

Anal. Calcd. for $C_{21}H_{24}N_2O_5$: C, 65.61; H, 6.29. Found: C, 65.79; H, 6.48.

Carbobenzyloxyglycyl-DL-phenylalanine ethyl ester was prepared in a similar manner but was obtained and used as an oil.

Carbobenzyloxy-DL-phenylalanylglycine (VIIIa).—The free acid was obtained by saponification of VIIIc with one equivalent of 2 *N* sodium hydroxide. After acidification with 3 *N* hydrochloric acid, the product separated as crystals in 93% yield, m.p. 150–157°. It was recrystallized from alcohol–water, melting at 152–154° and clearing at 159°.

Anal. Calcd. for $C_{19}H_{20}N_2O_5$: C, 64.03; H, 5.66. Found: C, 64.33; H, 5.81.

Carbobenzyloxyglycyl-DL-phenylalanine (Va).—The acid was prepared by saponification of the ester as described above for VIIIa. Recrystallized from alcohol, it melted at 158–160°, lit.[13] m.p. 156–157°.

Carbobenzyloxy-DL-phenylalanylglycineamide (VIIIb).—The ester VIIIc was added to 2.5 times its weight of methanol and the suspension was saturated with ammonia at 20°. The resulting solution was stored at room temperature for 3 days and concentrated under reduced pressure. The crude amide (85–90%) sintered *ca.* 120°, finally melting at 147°. After recrystallization from alcohol, it melted at 148–150° with resolidification and final melting *ca.* 190°. Resolidification was due to cyclization to the hydantoin amide, described below. Analysis indicated a mixture of amide and hydantoin containing 80–90% of VIIIb. Complete purification could not be achieved.

Carbobenzyloxyglycyl-DL-phenylalanineamide (Vb).—Ammonolysis of the ester was performed as described for VIIIa. The crystalline product sintered at 125° and melted at 158–160°. Crystallization from methanol yielded two forms: needles, sl. sol. in methanol, m.p. 161–162.5°; plates, sol. in methanol, m.p. 162–163°. When the needle-form was dissolved in methanol, the compound could be reisolated as plates; when the latter form was dissolved, needles were deposited.

Anal. Calcd. for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.83. Found: C, 64.26; H, 5.87; N, 11.51.

Carbonyl-N-DL-phenylalanineamide-N′-glycine Methyl Ester (VIIc).—Glycine methyl ester hydrochloride, 3.1 g. (0.025 mole) was added to a solution of 3.9 g. (0.05 mole) of pyridine in 10 ml. of methylene chloride. The suspension was chilled in ice and 7.8 g. (0.079 mole) of phosgene passed in (hood). The stoppered tube was brought to room temperature and the greenish-yellow suspension shaken occasionally. Within 30 minutes much of the solid had dissolved and after 1 hr. the solution was distilled until solution temperature reached 45°. Dry ether was added and the resulting solution was decanted from a crystalline mush. The residue was again washed with ether and filtered, and the combined extracts were concentrated to an oil weighing 1.6 g. (calcd. as 0.01 mole of the carbamyl chloride). To a solution of the oil in 7 ml. of methylene chloride was added 2.5 g. (0.01 mole) of DL-phenylalanineamide hydrobromide. To the chilled suspension was added 3.1 ml. of triethylamine. After 1 hr. at room temperature the solvent was removed, the residue triturated with dilute hydrochloric acid and the crystalline product filtered and washed with water. After trituration with alcohol the compound weighed 1.6 g. (56% based on carbamyl chloride) and melted at 176–178°. After purification from alcohol, the compound sintered at 160° and melted at 177–179°. When immersed in the bath at 170° the sample melted completely and then resolidified, suggesting dimorphism.

Anal. Calcd. for $C_{13}H_{17}N_3O_4$: C, 55.90; H, 6.14; N, 15.05. Found: C, 56.19; H, 5.91; N, 14.88.

Carbonyl-N-glycineamide-N′-DL-phenylalanine Methyl Ester (VIId).—The compound was prepared by the phosgene procedure described above from DL-phenylalanine methyl ester and glycineamide hydrochloride. The yield of material melting at 134–137° was 24%. After purification from 50% ethanol, it melted at 137–140°.

Anal. Calcd. for $C_{13}H_{17}N_3O_4$: C, 55.90; H, 6.14; N, 15.05. Found: C, 55.95; H, 5.94; N, 15.09.

This compound was also obtained by the esterification of the corresponding acid VIIb with ethereal diazomethane. It was not obtained as pure, m.p. *ca.* 135°, but its identity with the material previously prepared was confirmed by the infrared spectra.

Carbonyl-N-glycine-N′-DL-phenylalanine Diethyl Ester (VIIa).—The diester was prepared by the phosgene procedure described above from the individual ester hydrochlorides and by the action of ethanolic hydrogen chloride on the diacid VII. Purified from dilute ethanol, it melted at 107–109°.

Anal. Calcd. for $C_{16}H_{22}N_2O_5$: C, 59.61; H, 6.88; N, 8.69. Found: C, 59.41; H, 6.73; N, 8.78.

Carbonyl-N-glycine-N′-DL-phenylalanine (VII).—The diacid resulted from saponification of the isomeric amide-esters VIIc and VIId by heating on the steam-bath with a small excess of 2 *N* sodium hydroxide for 30 minutes. Acidification in both cases gave pure material, m.p. 171–172° (gas), in 92% yield.

Anal. Calcd. for $C_{12}H_{14}N_2O_5$: C, 54.13; H, 5.30; N, 10.52. Found: C, 54.01; H, 5.36; N, 10.38.

5-Benzylhydantoin-3-acetic Acid Ethyl Ester (IXc).—To a suspension of 1.0 g. of VIIIc in 1 ml. of abs. ethanol was added 0.2 ml. of 2.1 *N* sodium ethoxide in ethanol. The tube was stoppered with cotton and the contents stirred intermittently. After 40 minutes the separation of a crystalline product had created a very thick suspension, at which time the mixture was diluted with 2 ml. of ethanol. After 2 hr. 0.2 ml. of 3 *N* hydrochloric acid was added rapidly with stirring. The crystalline product was filtered and washed with alcohol; wt. 0.57 g. (79%), m.p. 147–151°. After purification from alcohol, it melted at 151–152.5°, lit.[5] m.p. 155°.

(11) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 225.

(12) Melting points are uncorrected.

(13) T. Wieland, W. Schäfer and E. Bokelmann, *Ann.*, **573**, 99 (1951).

Anal. Calcd. for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84. Found: C, 61.09; H, 5.94.

The ester was also prepared by the action of ethanolic hydrogen chloride on the corresponding hydantoin acid IXa.

5-Benzylhydantoin-3-acetic Acid (IXa).—A solution of 0.20 g. of the ester IXc in a small amount of ethanol was heated at reflux for 1 hr. with a solution of 0.5 g. of sodium bicarbonate in 4 ml. of water. Acidification yielded 0.14 g. (78%) of IXa, m.p. 180.5–182°. Hydrolysis with sodium hydroxide gave a less satisfactory yield (40%). It crystallized from alcohol as a mixture of prisms and needles, m.p. 180–182°, lit.[5] m.p. 181–183°.

IXa was also prepared by cyclization of the carbobenzyloxydipeptide acid. To a solution of 1.63 g. of VIIIa in 5 ml. of abs. ethanol was added 4.4 ml. (2 equiv.) of 2.1 *N* sodium ethoxide in ethanol. There occurred rapid formation of a gel. When all the starting material had dissolved, 3.5 ml. of 3 *N* hydrochloric acid was added rapidly with stirring. The solution was evaporated at room temperature on a watch glass, the yellow crystalline residue triturated with water and filtered. Purified from alcohol, it melted at 178–181°, wt. 0.81 g. (71%). Infrared diagrams of the two preparations were identical.

When IXa was dissolved in two equivalents of 2 *N* sodium hydroxide a sodium salt separated. After 5 hr. of intermittent shaking, it had redissolved. Upon acidification VII was isolated in good yield.

5-Benzylhydantoin-3-acetamide (IXb). Method A.—A crude preparation of VIIIb, 5.2 g., was suspended in 10.4 ml. of 2.1 *N* sodium ethoxide in ethanol. The solid dissolved in 15 minutes and, after standing an additional 30 minutes, the solution was added slowly with stirring to 8 ml. of cold 3 *N* hydrochloric acid. The crystalline product formed rapidly, 2.8 g. (77%), m.p. 210–213°. Recrystallized from alcohol it melted at 214–215°, lit.[5] m.p. 216–218°.

This cyclization could also be effected by means of heat. By heating VIIIb to 150–160° for 2–3 minutes, a 70% yield of IXb was obtained.

Method B.—When 0.1 g. of the ester-amide VIId was added to 0.4 ml. of methanol containing 0.1 ml. of triethylamine, solution occurred on heating. Within 1 minute heavy crystallization took place. After 4 minutes at reflux temperature the product was recovered, wt. 0.08 g., m.p. 214–215°.

Method C.—A sample of VIIb (*cf.* below), 0.36 g., was suspended in 2 ml. of abs. ethanol and hydrogen chloride gas added to saturation. After 30 minutes the solvent was removed under reduced pressure and the crude crystalline product purified from alcohol, wt. 0.17 g., m.p. 213–215°.

This cyclization could also be effected by heating the acid above its melting point. Held at 170° for 8 minutes, 0.10 g. yielded 0.05 g. of IXb, m.p. 210–214°.

The identity of the above samples was established by mixed melting point determinations.

Carbonyl-N-glycineamide-N′-DL-phenylalanine (VIIb).—This compound was isolated from the mother liquors resulting from the preparation of the hydantoinamide IXb from VIIIb. It was separated by means of its solubility in base. Less pure samples resulted from the hydrolysis of IXb with one equivalent of 2 *N* sodium hydroxide. Recrystallized from water, it melted at 168.5–170°.

Anal. Calcd. for $C_{12}H_{15}N_3O_4$: C, 54.33; H, 5.70; N, 15.84. Found: C, 54.30; H, 5.71; N, 15.58.

5-Cyclohexylmethylhydantoin-3-acetamide.—5-Benzylhydantoin-3-acetamide (IXb) in abs. ethanol with or without acetic acid absorbed hydrogen in the presence of Adams catalyst to give quantitatively a substance which no longer showed ultraviolet absorption for an aromatic ring and gave analytical values for the cyclohexane compound. Purified from alcohol it formed needles melting at 212.5–214° and depressed the melting point of IXb. The same product was isolated after hydrogenation in the presence of mineral acid, m.p. 215–216°.

Anal. Calcd. for $C_{12}H_{19}N_3O_3$: C, 56.90; H, 7.56. Found: C, 56.93; H, 7.56.

Hydantoin-3-DL-benzylacetic Acid (VIa).—Carbobenzyloxyglycyl-DL-phenylalanine (Va), 0.92 g., was dissolved in 3 ml. of abs. ethanol, and 2.5 ml. of 2.1 *N* sodium ethoxide in ethanol was added. After 4 hr. 3 ml. of 3 *N* hydrochloric acid was added rapidly with stirring. Four successive crops of crystals were obtained, the first three composed principally of starting material. The fourth crop weighed 0.13 g. and melted at 182–188°. Recrystallized from water, the product melted at 187–189°, lit.[9b] m.p. 190–193°.

Hydantoin-3-DL-benzylacetamide (VIb). Method A.—Carbobenzyloxyglycyl-DL-phenylalanineamide (Vb), 2.1 g., dissolved slowly in 4.2 ml. of 2.1 *N* sodium ethoxide in ethanol. After 2 hr. excess 3 *N* hydrochloric acid was added rapidly with stirring. The alcohol was evaporated and the crystalline residue freed of oil by washing with acetone. Recrystallized from water, it melted at 212–214°, wt. 0.095 g. (6.5%).

Method B.—When 1.3 g. of the amide-ester VIIc was added to 2.6 ml. of 2.1 *N* sodium ethoxide in ethanol, precipitation of a product occurred while the starting material was in the process of dissolving. The suspension was diluted with abs. ethanol and a slight excess of 6 *N* hydrochloric acid added. The filtered product weighed 1.03 g. (90%), m.p. 212–213.5°. Recrystallized from water it melted at 212–214°.

Anal. Calcd. for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 17.00. Found: C, 58.34; H, 5.28; N, 16.76.

5-Benzylhydantoin-3-acetonitrile (XIV).—5-Benzylhydantoin-3-acetamide (IXb), 2.0 g., was added to a mixture of 0.64 ml. (1 equiv.) of pyridine and 5 ml. of phosphorus oxychloride. The suspension was heated on the steam-bath until solution was complete and gas evolution had ceased (about 7 minutes). The phosphorus oxychloride was allowed to evaporate from a watch-glass, the residue triturated with water and the crystalline product filtered, wt. 1.48 g. (79%), m.p. 100–102°. Purified by recrystallization from dilute ethanol, it melted at 110–112° with a slight previous sintering.

Anal. Calcd. for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; N, 18.33. Found: C, 62.89; H, 4.82; N, 18.39.

When a solution of 0.075 g. of XIV in 0.5 ml. of concd. hydrochloric acid was shaken for 7 minutes and the crystalline residue obtained after concentration under reduced pressure was purified from ethanol, it melted at 213–214.5°, wt. 0.063 g., and was found to be identical with the amide IXb by mixed melting point determination.

Carbonyl-N-aminoacetonitrile-N′-DL-phenylalanine (XVI).—The hydantoinacetonitrile XIV, 1.48 g., was moistened with water and dissolved in 3.15 ml. (1.03 equiv.) of 2.12 *N* sodium hydroxide portionwise with stirring. Addition of a slight excess of 3 *N* hydrochloric acid precipitated an oil which crystallized, wt. 1.5 g. (94%), m.p. 151–153° (gas). Purified by recrystallization from water it melted at 155–157° (gas.).

Anal. Calcd. for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 17.0. Found: C, 58.48; H, 5.19; N, 16.5.

When XVI was treated with ethereal diazomethane and the product purified from alcohol, it melted at 109–112° and was identical with the hydantoin nitrile XIV. No product corresponding to the methyl ester of XVI could be isolated.

A sample of XVI, 0.1 g., dissolved in 0.5 ml. of concd. hydrochloric acid in 1 minute. After 5 minutes the solvent was evaporated, the residue triturated with water and filtered. The product melted at 168–170° (gas). After esterification with ethereal diazomethane, the compound did not depress the melting point of VIId.

1-Acetyl-5-benzylhydantoin-3-acetamide (XIII).—A solution of 0.40 g. of the hydantoinacetamide IXb in 1.2 ml. of pyridine was treated with 0.4 ml. of acetic anhydride and the mixture kept 2 hr. on a steam-bath. The solvent was removed under reduced pressure and the acetyl derivative crystallized upon addition of water, wt. 0.35 g. Purified from alcohol it sintered at 183° and melted at 187–189°.

Anal. Calcd. for $C_{14}H_{15}N_3O_4$: C, 58.12; H, 5.23; N, 14.53; CH_3CO, 14.88. Found: C, 58.03; H, 5.19; N, 14.04; CH_3CO, 14.19.

1-Acetyl-5-benzylhydantoin-3-acetonitrile (XV).—A solution of 0.11 g. of the nitrile XIV in 0.2 ml. of pyridine was treated with 0.1 ml. of acetic anhydride and the mixture kept on a steam-bath for 45 minutes. Solvent was removed under reduced pressure, the residue triturated with water and the crystalline product, 0.091 g., filtered. The crude product melted at 105–111°. After recrystallization from dilute alcohol, it melted at 112–115°.

A suspension of 0.10 g. of the amide XIII in 0.3 ml. of phosphorus oxychloride dissolved on the steam-bath with gas evolution. After 5 minutes heating, the acid chloride was allowed to evaporate on a watch-glass, the residue tri-

turated with water and the crystalline product filtered, 0.081 g., m.p. 103–108°. After recrystallization from alcohol it melted at 114–115°. The material did not depress the melting point of the acetyl derivative prepared from XIV.

Anal. Calcd. for $C_{14}H_{13}N_3O_3$: C, 61.98; H, 4.83, N, 15.49. Found: C, 62.00; H, 4.82; N, 15.38.

Hydantoin-3-DL-benzylacetonitrile (XVII).—The hydantoinamide VIb, 0.2 g., was added to a mixture of 0.064 ml. (1 equiv.) of pyridine and 0.5 ml. of phosphorus oxychloride. After 5 minutes on the steam-bath the solid dissolved and gas was eliminated. The acid chloride was allowed to evaporate, the residue triturated with water and filtered to give 0.177 g. of crystalline material (95%), m.p. 170–173°. Purified from alcohol it melted at 172–173.5°.

Anal. Calcd. for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; N, 18.33. Found: C, 63.20; H, 4.86; N, 18.30.

When XVII was dissolved in concd. hydrochloric acid and diluted with water after 6 hr., the starting material VIb was obtained, m.p. 209–211°.

Alkaline Rearrangement of XVII.—A suspension of 0.25 g. of XVII in alcohol was treated portionwise with one equivalent (0.52 ml.) of 2.12 *N* sodium hydroxide. Most of the solid had dissolved in 5 minutes with the concomitant separation of a sodium salt. After 10 minutes the crystalline mush was treated with 0.52 ml. of 2.1 *N* hydrochloric acid, resulting in complete solution. An additional 0.52 ml. of hydrochloric acid was added and the solution heated for 10 minutes. On cooling, 0.2 g. of prisms was obtained, m.p. 179–181.5°. The compound did not depress the melting point of 5-benzylhydantoin-3-acetic acid (IXa).

Alkaline Cyclization of XVI.—A suspension of 1.5 g. of XVI in 6 ml. of ethanol was treated with 4 ml. (1.4 equiv.) of 2.1 *N* sodium ethoxide in ethanol. The solid dissolved with simultaneous precipitation of a sodium salt. After 30 minutes the salt was filtered, weight 1.6 g. When an aqueous suspension of the sodium salt, 1.14 g., was treated with excess hydrochloric acid, an oil separated which soon crystallized, 0.68 g., m.p. 178–187°. Purified from ethanol it melted at 187–189° and was identical with VIa in melting point. For further confirmation, a sample was hydrolyzed by alkali at 25° to carbonyl-N-glycine-N′-phenylalanine (VII).

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BETHESDA 14, MARYLAND

[CONTRIBUTION FROM THE NUTRITION AND PHYSIOLOGY SECTION, RESEARCH DIVISION, AMERICAN CYANAMID CO., LEDERLE LABORATORIES]

The Synthesis of a Pteridine Required for the Growth of *Crithidia fasciculata*[1]

BY E. L. PATTERSON, R. MILSTREY AND E. L. R. STOKSTAD

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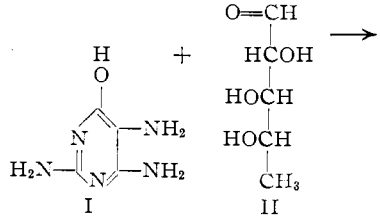
The condensation of 5-deoxy-L-arabinose with 2,5,6-triamino-4-hydroxypyrimidine sulfate yielded a mixture from which 2-amino-4-hydroxy-6-[1,2-dihydroxypropyl-(L-*erythro*)]-pteridine was isolated. The biological, chemical and physical properties of this compound were the same as those of biopterin, a pteridine isolated from human urine and required for the growth of *Crithidia fasciculata*.

In a previous communication[2] it was reported that a factor named biopterin required for the growth of *Crithidia fasciculata* had been isolated from human urine and characterized as 2-amino-4-hydroxy-6-(1,2-dihydroxypropyl)-pteridine. The optical configuration was not established. The details of the isolation and characterization are described in the accompanying paper.[3] In this paper the synthesis and some of the properties of 2-amino-4-hydroxy-6-[1,2-dihydroxypropyl-(L-*erythro*)]-pteridine are presented.

The usual method for the preparation of polyhydroxyalkyl pteridines of this type consists of the condensation of 2,5,6-triamino-4-hydroxypyrimidine with a sugar or sugar derivative[4–7] as shown. The yield of pteridine was not high, and two isomers having the alkyl side chain in the 6- and 7-positions were obtained starting with either an aldose, ketose or osone.[6] Hydrazine had some directive effect with the hexoses favoring the 6-isomer,[5] but little effect with pentoses.[5]



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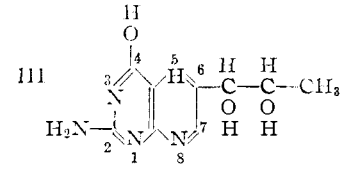

III

For the synthesis of the pteridine having the structure proposed for biopterin[2] by this method, the starting sugar should be a 5-deoxypentose having the proper optical configuration about carbon atoms three and four. Forrest and Mitchell[7] treated rhamnotetrose, prepared in solution, with 2,5,6-triamino-4-hydroxypyrimidine in the presence of hydrazine, and from the reaction mixture they obtained a product which was a mixture of 2-amino-4-hydroxy-6-(1,2-dihydroxypropyl)-pteridine and 2-amino-4-hydroxy-7-(1,2-dihydroxypropyl)-pteridine. By ultraviolet absorption and paper chromatography the synthetic preparation

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