ino acids remain stable for a long period, but N-carboxylic acid anhydrides are unstable in the presence of moisture and cannot survive storage, so that it is easier to copolymerize several sorts of amino acids. Some protein analogs were prepared by this method (Table I).

A detailed account of this work will be published in *J. Chem. Soc. of Japan*.

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RECEIVED MARCH 19, 1954

ISOLATION, STRUCTURE AND SYNTHESIS OF A LATHYRUS FACTOR FROM *L. ODORATUS*[1]

Sir:

The isolation from *Lathyrus odoratus* seeds of a crystalline substance capable of producing the skeletal abnormalities characteristic of lathyrism has recently been accomplished.[2-4] The substance (I) obtained in this Laboratory, m.p. 193–194° dec.,[5] was water-soluble, ninhydrin-positive, and gave analytical values agreeing with the formula $C_8H_{13}O_3N_3$.[3] It showed only one ninhydrin spot when subjected to paper chromatography in three different solvent systems; however, after hydrolysis in 11.7 *N* hydrochloric acid for 8 hours at 120° this spot disappeared and was replaced by two others. On concentrating and cooling the hydrolysis mixture, L-glutamic acid hydrochloride precipitated. This fragment was identified by m.p., ultimate analysis, infrared spectrum, optical rotation and microbiological assay. When the filtrate was made alkaline and distilled, a volatile base was evolved which was identified as ammonia by conversion to ammonium chloride and demonstrating the absence of carbon by the method of Pepkowitz.[6] This result together with a sharp band at 4.45 μ in the infrared spectrum pointed to the presence of a nitrile function in I. On this basis the remaining hydrolysis product could be only sarcosine, α-alanine or β-alanine. Comparative paper chromatograms clearly pointed to β-alanine as the actual degradation product, and its presence in the hydrolysate was verified by isolation of the β-naphthalene sulfonate of β-alanine,[7] m.p. 134–136°, both alone and mixed with an authentic sample. It was concluded that I is β-(γ-L-glutamyl)-aminopropiononitrile or the α-glutamyl isomer. The γ-glutamyl structure was favored because I showed pK values[3] of 2.2 ± 0.2 and 9.1 ± 0.1. Accordingly a substance of this structure was synthesized by the method of King and Kidd[8] by condensation of β-aminopropiononitrile with N-phthaloyl-L-glutamic anhydride and subsequent removal of the phthaloyl substituent with hydrazine. The synthetic product melted with decomposition at 193.5–194°[5] and showed no depression on admixture with isolated I. The identity of the two products was confirmed by a comparison of their infrared spectra which were alike in all respects. The lathyrus activity of the synthetic compound in rats is being investigated.

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RECEIVED APRIL 9, 1954

(1) Supported in part by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

(2) H. P. Dupuy and J. G. Lee, *J. Am. Pharm. Assoc.*, **43**, 61 (1954).

(3) G. F. McKay, J. J. Lalich, E. D. Schilling and F. M. Strong, *Arch. Biochem. Biophys.*, in press.

(4) E. D. Schilling, *Federation Proc.*, **13**, 290 (1954).

(5) Bath preheated to 180°.

(6) L. P. Pepkowitz, *Anal. Chem.*, **23**, 1716 (1951).

(7) H. H. Weinstock, H. K. Mitchell, E. F. Pratt and R. J. Williams, THIS JOURNAL, **61**, 1421 (1939).

(8) F. E. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949).

PHENYLACETYLGLUTAMINE AS A CONSTITUENT OF NORMAL HUMAN URINE

Sir:

Phenylacetylglutamine (PAG) was first described by Thierfelder and Sherwin[1] who isolated the compound from the urine of individuals fed phenylacetic acid. The conjugate is frequently referred to as a "detoxication" product. In investigating the source of the amino acids liberated by acid hydrolysis of human urine,[2] it has been found that PAG is excreted under normal conditions by the adult male to the extent of 250 to 500 mg. per day, and accounts for about 50% of the conjugated glutamic acid in urine.

Identification of PAG has been effected by chromatographic analyses of urine employing acidic and basic ion-exchange resins (Dowex 50 and Dowex 2). PAG and other conjugates were detected in the effluent by hydrolysis of 1-ml. effluent fractions with 6 *N* HCl or 2 *N* NaOH prior to the application of the photometric ninhydrin method.[3] Because of the affinity of the resin for aromatic compounds, PAG, despite its acidic nature, is retarded on columns of Dowex 50X8 and emerges at a position between urea and aspartic acid.[2] The conjugate was more readily determined by chromatography of 4-ml. samples of urine on 0.9 × 30 cm. columns of Dowex 2X4 (200–400 mesh) in the acetate form. Elution was begun with 0.2 *M* sodium acetate buffer at *p*H 5.3.[4] After 100 ml., the *p*H and ionic strength were gradually changed by allowing a 2 *M* sodium acetate buffer of *p*H 4.6[5] to flow into 100 ml. of the initial 0.2 *M* buffer stirred magnetically. The compound giving rise to a major peak at about 195 ml. was identified as PAG by the demonstration that the unknown and a sample of synthetic PAG[6] exhibited identical chromatographic behavior on columns of both Dowex 2 and Dowex 50, together with the finding of equimolar amounts of glutamic acid and ammonia in an acid hydrolysate of the unknown.

The same analytical procedure afforded a chro-

(1) H. Thierfelder and C. P. Sherwin, *Ber.*, **47**, 2630 (1914).

(2) W. H. Stein, *J. Biol. Chem.*, **201**, 45 (1953).

(3) S. Moore and W. H. Stein, *ibid.*, **176**, 367 (1948).

(4) 27.2 g. of $NaOAc \cdot 3H_2O$ + 5.0 ml. of glacial HOAc + 2.5 ml. of 50% BRIJ-35 solution diluted to 1 l. with water. BRIJ-35 is a detergent manufactured by Atlas Powder Co., Wilmington, Delaware.

(5) 54.4 g. of $NaOAc \cdot 3\ H_2O$ and 10.0 ml. of glacial HOAc are made to a volume of 100 ml. with water. To this solution 30 ml. of glacial HOAc, 70 ml. of water and 0.2 ml. of BRIJ-35 solution are added.

(6) H. Thierfelder and C. P. Sherwin, *Z. physiol. Chem.*, **94**, 1 (1915).

matographic determination of hippuric acid, which emerged at about 245 ml. from the Dowex 2 column. Normal adult males may excrete 1.0 to 2.5 g. of hippuric acid per day, a value which is higher than others previously reported and accounts for 65 to 75% of the observed conjugated glycine in 24 hour urines.[2] Hippuric acid and PAG together account for about half of the 2 g. of bound amino acids excreted daily.

The quantities of PAG and hippuric acid observed in the urines from fasting individuals, were similar to those reported above, indicating that both compounds probably are normal metabolic products, and do not arise only as a result of the "detoxication" of dietary precursors. In phenylpyruvic oligophrenia, PAG excretion was 2.4 g. per day (*cf.* 7) whereas the quantity of hippuric acid was diminished to about 0.3 g. In Wilson's disease, urinary hippuric acid was similarly reduced in amount but PAG excretion was normal.

We wish to acknowledge the technical assistance of Miss Joyce F. Scheer.

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RECEIVED APRIL 9, 1954

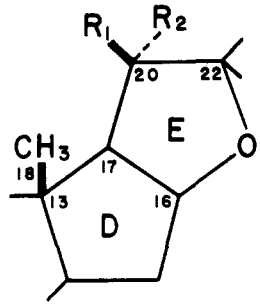
(7) L. I. Woolf, *Biochem. J.*, **49**, ix (1951).

(8) Rockefeller Foundation Fellow, 1951–1953.

STEROIDAL SAPOGENINS. XIX. STEREOCHEMISTRY OF SAPOGENINS AND CHOLESTEROL AT CARBON 20[1]

Sir:

The configuration of the methyl and hydrogen groups attached to the asymmetric C_{20} of steroid sapogenins has never been determined. We have established that naturally occurring sapogenins of both the 22b- and 22a-spirostane series have structure I at C_{20}. We have also prepared for the first time a new series of 20-isosapogenins with structure II. The evidence for these formulations follows. PSa[2,3] (m.p. 169–170°, $[\alpha]^{25}D$ +12°. Found: C, 77.79; H, 10.63) and PSm (m.p. 161°, $[\alpha]^{25}D$ +20°) on brief treatment at room temperature with alcoholic hydrochloric acid or 24 hours in ethanol–acetic acid form two new compounds which we have designated as 20-iSa and 20-iSm, respectively. The new compounds are isomeric with Sa and Sm; 20-iSa (m.p. 176–177°, $[\alpha]^{25}D$ +31.9°; Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.70; H, 10.62); 20-iSm (m.p. 185°, $[\alpha]^{25}D$ −60°; Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.92; H, 10.74). Refluxing 20-iSa and 20-iSm in alcoholic hydrochloric acid gave, respectively, Sa and Sm. Thus as we previously indicated[4] and later was shown by another group,[5] PSa and PSm are not identical as claimed by Marker and co-workers.[6] On acetylation at room temperature in pyridine–acetic anhydride, 20-iSa and 20-iSm both form monoacetates; 20-iSa acetate (m.p. 167°, $[\alpha]^{25}D$ +30°; Calcd. for $C_{29}H_{46}O_4$: C, 75.95; H, 10.11. Found: C, 75.94; H, 9.94); 20-iSm acetate (m.p. 160°, $[\alpha[^{25}D$ −49°; Calcd. for $C_{29}H_{46}O_4$: C, 75.95; H, 10.11. Found: C, 75.77; H, 10.05). Infrared spectra of both compounds showed a peak at 1732–1735 kr.[7] of strength corresponding to a monoacetate. Treatment of 20-iSa and 20-iSm with acetic anhydride at *reflux* or at 200° in a sealed tube resulted in smooth formation of PSa and PSm (after hydrolysis of the acetates).



I = Natural Sapogenins
R_1 = H, R_2 = CH_3

II = 20-Isosapogenins
R_1 = CH_3, R_2 = H

Fig. 1.—Configuration of natural and 20-isosapogenins at C_{20}.

Both 20-iSa and 20-iSm have complex infrared spectra in the region 650-1400 kr. associated with the spiroketal linkage at C_{22}.[8,9] The spectra of these two steroids are completely different from each other and also from Sa and Sm; among others 20-iSa has strong bands at 985, 965, 951, 917 and 905 kr.; 20-iSm at 974, 964, 920 and 897 kr.

Catalytic hydrogenation[10] of 20-iSa and 20-iSm resulted in formation of D20-iSa (m.p. 167°, $[\alpha]^{25}D$ −8°; Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.57; H, 10.98; diacetate, m.p. 96°, $[\alpha]^{25}D$ −3°; Calcd. for $C_{29}H_{50}O_5$: C, 74.06 H, 10.025. Found: C, 74.08; H, 10.17) and D20-iSm (m.p. 161°, $[\alpha]^{25}D$ +3°; Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.73; H, 11.02; diacetate, m.p. 96°, $[\alpha]^{25}D$ −4°; Calcd. for $C_{29}H_{50}O_5$: C, 74.06; H, 10.025. Found: C, 74.36; H, 10.09). As with DSa and DSm,[8,9] D20-iSa and D20-iSm do not have complex infrared spectra in the region 650–1400 kr. and have essentially identical spectra, which differ from that of DSa.[11] However, their respective X-ray diffraction powder patterns are completely different.

(1) Paper XVIII. M. M. Krider and M. E. Wall, THIS JOURNAL, **76**, in press (1954).

(2) Abbreviations used in this paper: Sa = sarsapogenin; Sm = smilagenin; P = pseudo; D = dihydro; 20-i = 20-iso. Thus PDSa = pseudodihydrosarsasapogenin.

(3) All melting points obtained with Kofler micro hot stage. Rotations in chloroform with exception of PSa and PSm which were in dioxane. Infrared spectra were obtained with CS_2 solvent.

(4) M. E. Wall, C. R. Eddy, S. Serota and R. F. Mininger, THIS JOURNAL, **75**, 4437 (1953).

(5) I. Scheer, R. B. Kostic and E. Mosettig, *ibid.*, **75**, 4871 (1953).

(6) R. E. Marker, *et al.*, *ibid.*, **61**, 3592 (1939); **62**, 648 (1940).

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(10) R. E. Marker and E. Rohrmann, *ibid.*, **61**, 846 (1939).

(11) We have found that compounds isomeric at carbon 25 cannot be distinguished by infrared spectra which are essentially identical. However, their X-ray diffraction patterns are markedly different. Compounds differing both at C_{22} and C_{25} can be distinguished by infrared spectra. Thus in the case of the infrared spectra of PSa, PSm; DSa, DSm; D20-iSa, D20-iSm; each pair has essentially identical spectra characteristically different from every other pair. Each individual compound has a characteristically different X-ray diffraction pattern. Full details of these findings will be presented in a detailed paper which will be submitted to THIS JOURNAL.