pool from which transformation products are formed.

The metabolism of cholesterol and its conversion to pregnenolone by adrenal homogenates have been consistently demonstrated in three additional experiments in approximately the same yield. The full details of these studies will be published subsequently. We should like to thank Dr. Harris Rosenkranz and Mr. Paul Skogstrom for the analysis and interpretations of the infrared spectra.

WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
SHREWSBURY, MASS.
N. SABA[7]
O. HECHTER
D. STONE

RECEIVED MAY 18, 1954

(7) Fellow of the Kellogg Foundation. On leave from the Ministry of Agriculture, England.

MASS SPECTRUM OF OCTABORANE

Sir:

From recent mass spectrographic studies[1] of various boron hydrides we have observed the mass spectrum of an octaborane, the existence of which was first postulated by Burg and Schlesinger[2] from vapor tension measurements.

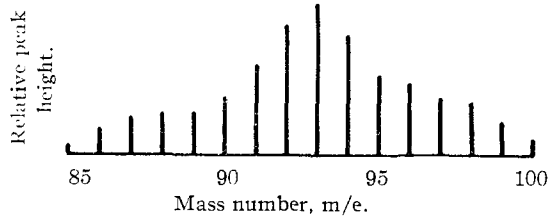

Fig. 1.—Partial mass spectrum of octaborane.

The mass spectrum of octaborane from mass numbers 85 to 100 is given in Fig. 1. The dominant peak occurs at mass number 93, and double ionization peaks are found in the region of mass numbers 44–48. The sharp cut-off in peak heights at mass number 100 suggests that the composition of the octaborane is B_8H_{12}, thus indicating that this compound belongs to the group of the (more) stable boron hydrides.[3]

In addition to the above findings we have been able to confirm Norton's finding of nonaborane.[4]

(1) A Consolidated Engineering Model 21-103 Mass Spectrometer operating at 70 volts was used in these studies.

(2) A. B. Burg and H. I. Schlesinger, THIS JOURNAL, **55**, 4009 (1933).

(3) E. Wiberg, *Ber.*, **69B**, 2816 (1936).

(4) F. J. Norton, THIS JOURNAL, **72**, 1849 (1950).

RESEARCH LABORATORY
MATHIESON CHEMICAL CORPORATION
PASADENA, CALIFORNIA
I. SHAPIRO
B. KEILIN

RECEIVED JUNE 17, 1954

BIOSYNTHESIS OF OROTIC ACID FROM CITRULLINE

Sir:

Orotic acid, a pyrimidine precursor in bacteria[1] and in the rat,[2] is believed to arise from aspartate via ureidosuccinate and dihydroörotate. Ureidosuccinate has been pictured as arising from arginosuccinate, based on the finding that the ureide carbon of citrulline[3] as well as carbon dioxide[4] contribute to position-2 of tissue pyrimidines in the pigeon. In the rate and in rat liver homogenates, no such incorporation from citrulline could be demonstrated,[3,5] and this failure was attributed to active degradation of citrulline to urea in mammalian liver. In the present study, pigeon and rat liver slices have been compared in regard to incorporation into orotic acid of the ureide carbon of citrulline and the amidine carbon of arginine.

L(+)-Citrulline was prepared from urea-C^{14} by the method of Kurtz.[6] L(+)-Arginine·HCl was synthesized from cyanogen bromide-C^{14} via O-methylisouronium chloride.[6] Both materials had specific activities of 2.84×10^6 c.p.m. per mmole. Incubation was carried out essentially according to Reichard[7] using 15–18 g. of pigeon or rat liver slices, 50 ml. of Krebs–Henseleit bicarbonate medium supplemented with 90 mg. of glucose, 50 mg. of sodium ATP and 15 mg. of carrier orotic acid. Each bath also contained either 0.1 mmole of L-citrulline-C^{14} + 0.5 mmole of L-aspartate or 0.1 mmole of L-arginine·HCl-C^{14} + 0.5 mmole of fumarate. After 4 hours of incubation at 37°, orotic acid was recovered from each deproteinized medium as described by Reichard.[7] The product was characterized by ultraviolet absorption spectrum and m.p. (341–343°) after recrystallization from water. All samples were combusted, and counted as barium carbonate under a thin-window Geiger–Mueller counter.

TABLE I

RADIOACTIVITY OF ISOLATED OROTIC ACID FROM LIVER SLICE STUDIES

Substrate	Rat liver, c.p.m. per milliatom C	Pigeon liver, c.p.m. per milliatom C
L(+)-Citrulline-C^{14}	3,390	0
L(+)-Citrulline-C^{14}	3,040	0
L(+)-Arginine-C^{14}	0[a]	0
L(+)-Arginine-C^{14}		0

[a] Zero means <3 c.p.m. above background of *ca.* 25 c.p.m.

In contrast to expectations based upon the work of others cited above, significant incorporation of ureide carbon of citrulline into orotic acid was observed with rat but not with pigeon liver slices (Table I). An apparent paradox, as yet unresolved, arises from the demonstration that in the rat citrulline → orotic acid (present study), orotic acid → pyrimidines,[8] yet citrulline failed to contribute specifically to pyrimidines.[3,5] In the pigeon no contribution from citrulline to orotic acid was detected (present study) yet citrulline has been shown to contribute to pyrimidines.[3] The present findings complement the earlier ob-

(1) L. D. Wright, C. S. Miller, H. R. Skeggs, J. W. Huff, L. L. Weed and D. W. Wilson, THIS JOURNAL, **73**, 1898 (1951).

(2) H. Arvidson, N. A. Eliasson, E. Hammarsten, P. Reichard, H. von Ubich and S. Bergstrom, *J. Biol. Chem.*, **179**, 169 (1949).

(3) M. P. Shulman and S. J. Badger, *Federation Proc.*, **13**, 292 (1954).

(4) M. R. Heinrich and D. W. Wilson, *J. Biol. Chem.*, **186**, 447 (1950).

(5) C. Cooper and D. W. Wilson, *Federation Proc.*, **13**, 194 (1954).

(6) A. C. Kurtz, *J. Biol. Chem.*, **122**, 477 (1937–38), and **180**, 1253 (1949).

(7) P. Reichard, *J. Biol. Chem.*, **197**, 391 (1952).

(8) L. L. Weed and D. W. Wilson, *J. Biol. Chem.*, **189**, 435 (1951).

servations of Reichard,[9] and together with these, delineate the probable pathway of orotic acid synthesis in the rat: citrulline + aspartate → (arginosuccinate) → ureidosuccinate → (dihydroorotate) → orotic acid.

Neither in rat nor in pigeon liver slices (Table I) could any contribution to orotic acid from the amidine carbon of arginine be detected. The postulated intermediate arginosuccinate would therefore appear not to arise from arginine in the preparations studied.

(9) P. Reichard and U. Lagerkvist, *Acta Chem. Scand.*, **7**, 1207 (1953).

DIVISION OF NUTRITION AND PHYSIOLOGY
THE PUBLIC HEALTH RESEARCH INSTITUTE L. H. SMITH, JR.
OF THE CITY OF NEW YORK, INC. DEWITT STETTEN, JR.
NEW YORK, N. Y.

RECEIVED JUNE 15, 1954

THE STEREOCHEMISTRY OF STEROIDAL SAPOGENINS

Sir:

Despite the voluminous literature[1] dealing with the structure and chemistry of the steroidal sapogenins, the stereochemistry of the spiroketal side chain of these substances remains obscure. In light of recent discoveries this appears to have been due in large part to the failure to appreciate the importance of the asymmetry at *both* the C-22 and C-25 positions. The fact that certain of these substances can be converted[2] by rather vigorous acid treatment into isomeric substances, together with other lines of evidence,[3] has led to the assumption[3] that such naturally occurring stereoisomeric pairs of steroidal sapogenins as sarsasapogenin and smilagenin are epimeric at C-22. Recent studies[4,5] of infrared absorption have shown that the spectra of all naturally occurring steroidal sapogenins thus far examined possess bands in the vicinity of 980, 920, 900 and 860 cm.$^{-1}$, which are characteristic of the spiroketal side chain. The relative intensities of the 920 and 900 cm.$^{-1}$ bands have been correlated with the so-called "normal" and "iso" series, which were assumed to be epimeric at C-22. However, recent work[6] involving careful repetition of some earlier transformations together with degradation studies has demonstrated that sarsasapogenin and smilagenin are epimeric at C-25 and does not exclude the possibility that these substances actually have the same configuration at C-22. By means of similar degradation of diosgenin and hecogenin and by correlation of side chain degradation products with the absolute configuration of D-glyceraldehyde, James[7] has shown that diosgenin, hecogenin and smilagenin all have the D-configuration at C-25 while sarsasapogenin has the L-configuration. The configuration at C-22 (spiroketal) together with previous correlations[4,5] of infrared absorption with side-chain structure therefore comes into question.

We have converted pseudodiosgenin[8] [m.p. 165–168°, $[\alpha]^{25}D$ −39° (1% in chf.); found: C, 78.26; H, 9.91; medium band in infrared[9] at 1693 cm.$^{-1}$ (1% in chf.)] into neodiosgenin[2b] [m.p. 197–201°, $[\alpha]_D$ −122° (1% in chf.); found: C, 77.77; H, 9.84; shoulder in infrared at 982 cm.$^{-1}$, strong bands at 960, 920, 896 cm.$^{-1}$, weak bands at 856 and 973 cm.$^{-1}$ (1% in CS_2), 896 cm.$^{-1}$ band more intense than 920 cm.$^{-1}$ band. Mixture melting point with diosgenin (m.p. 208–211°), m.p. 191–192.5°] by very mild treatment with acid. Upon more vigorous acid treatment, neodiosgenin yielded diosgenin[4] [m.p. 206–211°, no depression mixed with an authentic specimen, strong bands in infrared at 982, 963, 921 and 900 cm.$^{-1}$, weak band at 860 cm.$^{-1}$; 982 cm.$^{-1}$ band more intense than 963 cm.$^{-1}$ band, 900 cm.$^{-1}$ band more intense than 921 cm.$^{-1}$ band].

Consideration of the probable mechanism of conversion of pseudodiosgenin to neodiosgenin and this to diosgenin leads to the assignment of structure II to neodiosgenin and structure III to diosgenin. These acid-catalyzed reactions are conceived to be polar in nature, with *trans* addition occurring across the C-20,C-22 double bond.[10] In the initial cyclization of pseudodiosgenin to neodiosgenin, it would appear that attack of a solvated proton at C-20 from the less hindered rear face of the molecule is kinetically favored and leads to structure II. However, on prolonged treatment with acid a process of equilibration leads to the thermodynamically more stable structure III; the driving force for the conversion of neodiosgenin (II) to diosgenin (III) is furnished by hindrance between the C-18 methyl groups and C-21 and possibly by the less stable axial conformation[11,12] of the C-27 methyl group in structure II. The initial formation of the kinetically favored but thermodynamically less stable neodiosgenin (II) and its subsequent double inversion at C-20 and C-22 to the more stable diosgenin (III) is analogous to the formation of cholesterol 5α,6β-dibromide and its isomerization to the more stable 5β,6α-dibromide.[13]

It will be noted that the relative intensities of the infrared bands near 900 cm.$^{-1}$ and 920 cm.$^{-1}$ are of

(1) See L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publ. Corp., New York, N. Y., 1949, Chapt. VIII.

(2) See, for example: (a) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 846 (1939); (b) R. E. Marker and J. Lopez, *ibid.*, **69**, 2373 (1947).

(3) See ref. 1, pp. 587, 589.

(4) C. R. Eddy, M. E. Wall and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953).

(5) R. N. Jones, E. Katzenellenbogen and K. Dobriner, *ibid.*, **75**, 158 (1953).

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

(7) V. H. T. James, *Chemistry and Industry*, 1388 (1953).

(8) (a) R. E. Marker, T. Tsukamoto and D. L. Turner, THIS JOURNAL, **62**, 2525 (1940). (b) D. H. Gould, H. Staeudle and E. B. Hershberg, *ibid.*, **74**, 3685 (1952).

(9) A. L. Hayden, P. B. Smelzer and I. Scheer, *Anal. Chem.*, **26**, 550 (1954).

(10) The C-20,C-22 double bond of pseudodiosgenin requires the *cis* fusion of rings D and E on steric grounds, presumably in the β-configuration.

(11) The axial conformation of the C-27 methyl group coincides with hindrance between the C-18 and C-21 methyl groups *only* if the absolute configuration of the steroid nucleus is as drawn; cases II and III would then represent absolute configurations of neodiosgenin and diosgenin, respectively. If the absolute configuration of the steroid nucleus is actually the mirror image of its usual representation, neodiosgenin (II) would differ from the above structure in possessing an equatorial methyl group at C-25, and diosgenin (III) would then have the axial methyl group at C-25.

(12) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).

(13) D. H. R. Barton and E. Miller, THIS JOURNAL, **72**, 1066 (1950).