This investigation of the dehydrohalogenation of neoalkyl halides by strong base was made to elucidate the reaction mechanism. From the experimental data it can be concluded that carbanions and/or strong bases attack and remove an α-hydrogen atom with displacement of halide (*α-elimination*) to form the carbene intermediate which gives *cyclopropanes* by *intramolecular-insertion*[4a,c] and *olefins* by *rearrangement*.[4]

Thus, neopentyl halides and sodium, sodamide in diethyl Carbitol, or phenyl sodium in decane give 1,1-dimethylcyclopropane, small amounts of 2-methyl-2-butene, neopentane[5] and corresponding substitution products (Table I).

TABLE I

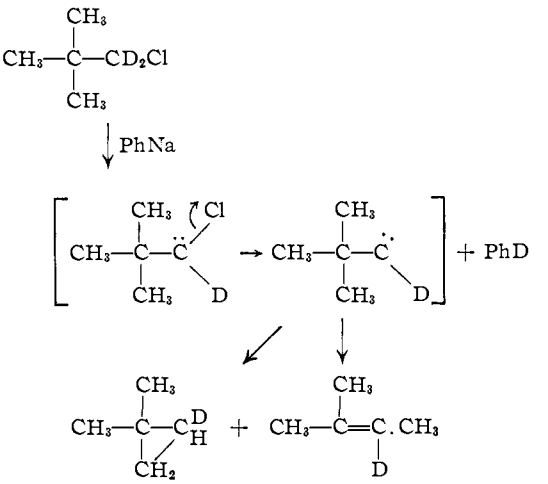
REACTION OF NEOPENTYL HALIDES WITH STRONG BASES

		Dehydrohalogenation products[a,b]			
X	Base	Neopentane	1,1-Dimethylcyclopropane	2-Methyl-2-butene	Yield[c]
Cl-1-d_2	PhNa[d]	14[e]	82	4.3	71
Cl	PhNa	9.2	87	3.5	70
Cl	$NaNH_2$	6.9	90	2.9	60
Cl	Na[f]	5[f]	82	7	60
Br	PhNa	5.5	86	7.3	25
DMPT	NaOMe	...	89	9	85][g]

[a] % composition. Products analyzed, separated and identified by v.p.c. [b] Traces (<0.1%) of 2-methyl-1-butene also present, presumably from base-catalyzed isomerization of 2-methyl-2-butene. [c] Conversion of neopentyl halides 100%. SN2 products account for the balance. [d] Reaction conditions: 10% excess base; PhNa suspension in decane (100°), $NaNH_2$ in diethyl Carbitol (reflux, *ca.* 180°), Na, no solvent (Wurtz reaction). [e] Increased amount apparently due to primary isotope effect. [f] Product composition corrected for large amount (110% of theory) of neopentane formed as a result of neopentyl sodium functioning as the base. [g] 2,2-Dimethylpropanal (pivaldehyde) tosylhydrazone treated with NaOMe in diethyl Carbitol at 180°. 2-Methyl-1-butene (2%) also formed.

Product composition and yields compare closely with those obtained from thermal decomposition of 2,2-dimethylpropanol tosylhydrazone anion in aprotic media (Table I), a reaction which proceeds via the alkyl carbene.[4a] In addition, the same carbene generated by an independent method gives these products in similar yield.[4c] This suggests that the dehydrohalogenation proceeds through the same intermediate.

To test this hypothesis, 1,1-dideuteroneopentyl chloride was treated with phenyl sodium to give dehydrohalogenation products and benzene which were analyzed for deuterium content.[6] 1,1-Dimethylcyclopropane and 2-methyl-2-butene formed contained *only one* deuterium/molecule. Deuterobenzene also was obtained.[7] These results support the proposed mechanism of the reaction.



Neohexyl chloride (1-chloro-2,2-dimethylbutane) reacts with sodium or sodamide in diethyl Carbitol to give dehydrohalogenation products consistent with the proposed mechanism, *i.e.*, 1-ethyl-1-methylcyclopropane (61, 67)[8] and 1,1,2-trimethylcyclopropane (32, 34) via *carbene insertion* and olefins: 2-methyl-2-pentene (0.7, 1.3), *cis*-3-methyl-2-pentene (1.4, 3.1) and *trans*-3-methyl-2-pentene (3.0, 4.2), via *carbene rearrangement*. In addition, thermal decomposition of 2,2-dimethylbutanal tosylhydrazone anion in aprotic media yields the same products in similar amounts.

These results are not in agreement with those reported previously.[2c,9] Part of the discrepancy can be attributed to the purity of the neohexyl chloride employed.[2c]

(7) Isolated yield of C_5 hydrocarbons 71%, deuterobenzene 76%.

(8) Product composition (%) from reaction with sodium and sodamide, respectively; adjusted for the neohexane formed.

(9) 1,1,2-Trimethylcyclopropane, neohexane and *tert*-butylethylene were obtained to the complete exclusion of 1-ethyl-1-methylcyclopropane.

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1555 (1958); Abstracts of Papers, 137th Meeting of the American Chemical Society, Cleveland, Ohio, April, 1960, p. 10-O.

(4) (a) L. Friedman and H. Shechter, *J. Am. Chem. Soc.*, **81**, 5512 (1959). (b) *Ibid.*, **82**, 1002 (1960). (c) G. L. Closs, Abstracts of Papers, 138th Meeting of the American Chemical Society, New York, N. Y., Sept., 1960, p. 9-P. (d) Neophyl chloride (1-chloro-2-methyl-2-phenylpropane) reacts with strong bases to give products consistent with the carbene mechanism. *Cf.* F. C. Whitmore, C. A. Weisgerber and A. C. Shabica, *J. Am. Chem. Soc.*, **65**, 1469 (1943).

(5) Formed by halogen-metal interchange and subsequent hydrogen abstraction.

(6) By mass spectrographic analysis. We wish to acknowledge the assistance of Messrs. S. Zolty and R. O. Henselman, Instrumentation Laboratory, Research Division, College of Engineering, New York University.

CHEMICAL STRUCTURE OF A PHOSPHOMUCOLIPID AND ITS OCCURRENCE IN SOME STRAINS OF SALMONELLA[1]

Sir:

Extraction of some gram negative bacteria with phenol[2,3,4] produces a water-soluble lipopolysaccharide complex containing bound amino acids. This can be split into water-insoluble lipid and degraded polysaccharide by dilute acetic acid,[5] hydrochloric acid,[4] or by treatment with cationic ion exchangers (unpublished observations).

(1) The Salmonella strains were kindly supplied to us by Drs. R. List and E. Ribi of the Public Health Service's Rocky Mountain Laboratory, Hamilton, Montana.

(2) J. W. Palmer and T. D. Gerlough, *Science*, **92**, 155 (1940).

(3) W. T. J. Morgan and S. M. Partridge, *Biochem. J.*, **35**, 1140 (1941).

(4) O. Westphal and O. Lüderitz, *Angew. Chem.*, **66**, 407 (1954).

(5) G. G. Freeman, *Biochem. J.*, **36**, 340 (1942).

TABLE I

Prepn.	Hydrolysis	Reaction: Elson–Morgan	Reaction: Reducing aldehyde	Reaction: Consumed IO_4^-	Percentage: C	H	N	P	NH_2	Ratios: N/NH_2	N/P
GAP-I	Before	Pos.	1 mole	4 moles	27.60	5.38	5.50	11.72	6.01	~1	~1
	After	Pos.	1 mole	<5 moles							
GAP-II	Before	Neg.	1 mole	3 moles	27.90	5.70	5.48	12.02	6.12	~1	~1
	After	Pos.	1 mole	<5 moles							
GAP-III	Before	Neg.	Neg.	...	38.12	5.42	10.89	5.96	7.07	~4/2	~4/1
	After	Pos.	Pos.	...							

This lipid mixture, called "Lipid-A" by Westphal and Lüderitz,[4] has been fractionated[6] and the chemical structure of one of the purified components, which appears to be a paper chromatographically homogenous split-product of the lipopolysaccharide, was examined in some *E. coli* strains.[7]

During recent investigations, lipopolysaccharide also has been isolated using Goebel's modified phenol method[8] from *S. gallinarium 1X, XII, S. typhosa Ty-2, S. typhosa 0901, S. typhimurium, S. enteritidis 795*, and *S. oranienburg 1687T* samples. The chemical structure of the isolated, purified lipid has been investigated in all six, most extensively in the first two strains. In view of the content of phosphorus (1.9–2.2%), glucosamine (18.1–20.5%), and fatty acid (50–55%), this preparation was called a phosphomucolipid.

Components of the Lipid.—Approximately 65% of the dry weight became ether-soluble and about 50% water-soluble after 10 hours of hydrolysis with 3*N* HCl in boiling water-bath. The extra 15% recovered was attributable to the water taken up during hydrolysis.

Eighty-six per cent. of the ether-soluble components consisted of free fatty acid, determined titrimetrically and calculated as palmitic acid. Paper chromatographic analysis of the extract showed the same fatty acids as are found in the lipopolysaccharide.[9]

The water-soluble hydrolysate analyzed by high-voltage paper electrophoresis was found to contain a large amount of glucosamine, which, when isolated, was shown to be dextrorotatory.[10] Specific rotation is $[\alpha]^{20}D$ +46.7°. Considerably smaller amounts of aspartic acid, glutamic acid, alanine, serine, valine, arginine, and lysine, and three ninhydrin-positive compounds not identical with known amino acids also were detected.

These unknown ninhydrin-reactive components gave a positive test with the Trevelyan polyol specific reaction,[11] and with the Hanes and Isherwood phosphoric ester reagent.[12] A weak reaction was given with the Elson–Morgan reagent on filter paper.[13] These components were isolated by ion exchange chromatography on Dowex 50, two of them in crystalline form. Total hydrolysis in each instance yielded D-glucosamine and phosphoric acid, showing the three compounds to be D-glucosamine-phosphate derivatives (GAP-I, GAP-II, GAP-III). One of the compounds (GAP-III) contained all the amino acids present in the total hydrolysate of the lipid, indicating it to be a peptide containing D-glucosamine-phosphate.[6]

The number of reducing groups was determined.[14] Quantitative studies were made of the periodate oxidation[15] and the Elson–Morgan reaction.[16] Results showed the compounds to be 6-phospho-D-glucosamine (GAP-I), 4-phospho-D-glucosamine (GAP-II), and 1-peptido-4-phospho-D-glucosaminide (GAP-III).[7,10] Some analytical data are given in Table I. Detailed descriptions of these experiments will be published elsewhere.

Ratios and Linkages between the D-Glucosamine Phosphate Derivatives.—The sequence was followed of appearance and disappearance of the hydrolysis split products obtained with dilute HCl. The acid-stable 6-phospho-D-glucosamine appeared coincidentally with the disappearance of the acid-labile 4-phospho-D-glucosamine and its peptide derivative. The 6-phospho-D-glucosamine is produced during acid hydrolysis by transphosphorylation from the two 4-phospho-D-glucosamine derivatives.[7] These two derivatives and D-glucosamine were liberated by hydrolysis in approximately isomolar amounts.

Two possibilities exist for the linkage between the D-glucosamine-phosphate units: (a) direct linkages or (b) linkage through phosphodiester bridges, as in the case of teichoic acid, which is present in the cell wall of some gram positive bacteria.[17]

Alkaline and acid phosphatases failed to split free phosphoric acid from the lipids. Results with venom diesterase, which made the existence of a phosphodiester linkage between the glucosamine units seem likely, will be published shortly.

Position of the Fatty Acids.—Glycerol and sphingosine were not detected in this lipid. Hydrazinolysis showed the amino group of the glucosamine to be acylated, approximately 30% of the total fatty acids being bound to amino groups. A portion of the fatty acids (35–45%) are bound through labile ester linkages probably to the C-3 hydroxyl groups of the glucosamine, as shown by deacylation with 0.01 *N* $NaOCH_3$ in a water-free

(6) A. Nowotny, E. Eichenberger, H. Hurni, O. Lüderitz and O. Westphal, IV Int. Congr. Biochem., Vienna 1958. German patent applied for (W 23784 IVa/30h, July 25, 1958); U. S. patent applied for (829,485, July 27, 1959).

(7) A. Nowotny, Conference on Biological Effects of Endotoxins in Relation to Immunity, Freiburg, Germany, 1959.

(8) W. F. Goebel and G. T. Barry, *J. Exp. Med.*, **107**, 185 (1957).

(9) A. Nowotny, O. Lüderitz and O. Westphal, *Biochem. Z.*, **330**, 47 (1958).

(10) A. Closse, Doctoral thesis, University of Freiburg, Germany, 1960.

(11) W. E. Trevelyan, D. C. Procter and J. S. Harrison, *Nature*, **166**, 444 (1950).

(12) C. S. Hanes and F. A. Isherwood, *ibid.*, **164**, 1107 (1949).

(13) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(14) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

(15) E. A. Adelberg, *Anal. Chem.*, **25**, 1553 (1953).

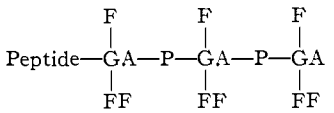
(16) R. Kuhn, A. Gauhe and H. H. Baer, *Chem. Ber.*, **87**, 1138 (1954).

(17) J. J. Armstrong, J. Baddiley, J. G. Buchanan, B. Carss and G. R. Greenberg, *J. Chem. Soc.*, 4344 (1958); *Biochem. J.*, **76**, 610 (1960).

medium at room temperature.[10,18] The remainder probably is bound to C-6, forming a relatively stable substituent. The investigation of the exact position of the different fatty acids is not complete.

In addition to the six Salmonella bacteria previously mentioned, the hydrolysate of similarly isolated lipids from *Serratia marcescens*, *Pseudomonas aeruginosa*, *E. freundii*, *E. coli 055* and *Neisseria gonorrhoeae*[19] also contained 4-phospho-D-glucosamine derivatives.

This phosphomucolipid is thus a new lipid containing a poly-D-glucosamine-phosphate chain, in which the glucosamine is esterified with fatty acids. The tentative structure is



F, fatty acid; GA, D-glucosamine; P, phosphoric acid.

(18) G. Zemplén, A. Gerecs and J. Hadacsy, *Chem. Ber.*, **69B**, 1827 (1936).

(19) The purified *Neisseria gonorrhoeae* lipopolysaccharide was kindly supplied by Dr. H. Tauber, Public Health Service's Venereal Disease Experimental Laboratory, Chapel Hill, N. C.

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STRUCTURE AND ENZYMATIC CLEAVAGE OF AGARITINE, A PHENYLHYDRAZIDE OF L-GLUTAMIC ACID ISOLATED FROM *AGARICACEAE*[1]

Sir:

From the press-juice of *Agaricus bisporus*[2] a new derivative of glutamic acid has been isolated and characterized as β-N-(γ-L(+)-glutamyl)-4-hydroxymethylphenylhydrazine. Separation and purification of this compound (herein designated by the trivial name, agaritine) was achieved by ion-exchange chromatography on Dowex 1 acetate resins and descending, preparative chromatography on sheets of Whatman no. 17 paper developed with 1-butanol–acetic acid–water [4:1:5] followed by rechromatography on Whatman no. 3 paper with 70% ethanol. Agaritine was obtained from 93% ethanol as a colorless, neutral, microcrystalline solid, $[\alpha]^{23}D$ +26.2° (0.65% in water), decomposition range (without melting) 203–208°, molecular weight[3] 262, calculated for $C_{12}H_{17}N_3O_4$ (mol. wt., 267): C, 53.92; H, 6.41; N, 15.72; O, 23.95; Found: C, 53.89; H, 6.48; N, 15.54; O, 24.38. Although extremely soluble in water, it does not dissolve in any of the common anhydrous organic solvents. Solutions of the compound exhibit a characteristic ultraviolet absorption spectrum ($\lambda_{max.}$ = 237.5 mu and 280 mu; ϵ = 11,400 and 1200, respectively) that remains unaffected by changes in hydrogen ion concentration between *p*H 2 and *p*H 12.

In Table I are presented the results of a variety of group analyses performed on agaritine.

(1) Initial phases of this investigation have been reported in abstract form [B. Levenberg, *Federation Proc.*, **19**, 6 (1960)].

(2) The mushroom of commerce in the United States. Source of supply for these studies was the Michigan Mushroom Co., Niles, Michigan.

(3) Determined by thermoelectric osmometry.

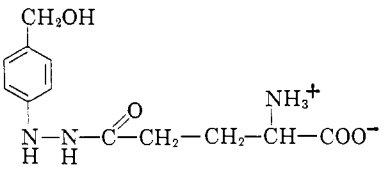
TABLE I

FUNCTIONAL GROUP ANALYSES OF AGARITINE

Determination	Moles per mole of agaritine ($C_{12}H_{17}N_3O_4$)
α-Amino group[a]	0.90
α-Carboxyl group[b]	1.05
L-Glutamic acid[c]	0.96[d]
	0.95[e]
Aryl hydrazine[f]	0.99[g]
Reducing group[g]	1.00
Ninhydrin chromophore[h]	0.87
Potentiometric titration[i]	
Acidic dissociation (*pK* 2.5)	0.96
Basic dissociation (*pK* 9.1)	0.93

[a] Performed by Huffman Microanalytical Laboratories, Wheatridge, Col. [b] By the ninhydrin decarboxylation method of D. D. Van Slyke, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, 141, 671 (1941). [c] By the enzymatic method of N. O. Kaplan, M. M. Ciotti and F. E. Stolzenbach, *ibid.*, 221, 833 (1956). [d] After acid hydrolysis. [e] After enzymic hydrolysis. [f] By spectrophotometric titration with crystalline sodium pyruvate at 320 mu. [g] By the method of O. Folin and V. Ciocalteu, *J. Biol. Chem.*, **73**, 627 (1927). [h] Using L-glutamine as a standard.[4] [i] Performed by discontinuous titration in aqueous media.

The substance produces a violet chromophore with the ninhydrin reagent[4] typical of those formed by many of the common α-amino acids. Cleavage of agaritine with hot, dilute hydrochloric acid (or by action of the partially-purified enzyme described below) results in the liberation of L-glutamic acid and a second compound that can be oxidized with selenous acid at 15° to produce an aryldiazonium salt.[5] On mild acid hydrolysis, the latter yields a single phenol that has been identified by paper chromatographic techniques[6] as 4-hydroxybenzyl alcohol, through comparison with an authentic sample and by conversion to the 4-hydroxyphenylazobenzene *p*-sulfonic and *o*-carboxylic acid derivatives.[7] Together with the elemental and functional group analyses reported above, these degradational observations indicate this structure for agaritine



Agaritine, β-N-(γ-L(+)-glutamyl)-4-hydroxymethylphenylhydrazine

The reducing property, which is manifested by the ready, irreversible oxidation of agaritine with reagents such as neutral ferricyanide, alkaline Ag^+, and periodate, is seen to reside in the di-substituted hydrazine function.[8] This structure appears unique in being the first example of its kind to be reported in natural products.

A highly active enzyme, purified some 30-fold from soluble extracts of *A. bisporus*, catalyzes cleav-

(4) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

(5) F. Feigl, "Spot Tests in Organic Analysis," Elsevier Publishing Co., Amsterdam, 1956, pp. 294–296.

(6) J. H. Freeman, *Anal. Chem.*, **24**, 955 (1952).

(7) W. H. Chang, R. L. Hossfeld and W. M. Sandstrom, *J. Am. Chem. Soc.*, **74**, 5766 (1952).

(8) An aberrant course of oxidation is observed in hot, alkaline permanganate solution, resulting in the formation, in quite small yield, of a mixture of benzoic and terephthalic acids.