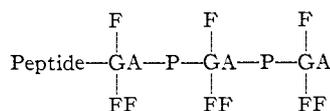medium at room temperature.[10,18] The remainder probably is bound to C-6, forming a relatively stable substituent. The investigation of the exact position of the different fatty acids is not complete.

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

$$
\begin{array}{c}
\text{F} \quad\quad \text{F} \quad\quad \text{F} \\
| \quad\quad | \quad\quad | \\
\text{Peptide—GA—P—GA—P—GA} \\
| \quad\quad | \quad\quad | \\
\text{FF} \quad\quad \text{FF} \quad\quad \text{FF}
\end{array}
$$

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 re-chromatography 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 pH 2 and pH 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[e]
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-hydroxyphenyl-azobenzene 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
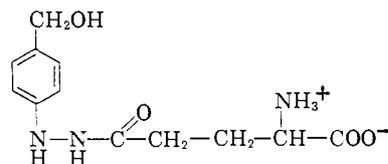
Agaritine, β-N-(γ-L.(+)-glutamyl)-4-hydroxymethylphenylhydrazine

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.

age of agaritine to 4-hydroxymethylphenylhydrazine and L-glutamic acid. In the presence of an excess of glyoxylic or certain other α-keto acids, the hydrolysis may be followed spectrophotometrically by observing the rate of formation of the corresponding phenylhydrazone derivatives, which exhibit absorption maxima in the region of 325 mu. A variety of β-N-acylphenylhydrazine analogs have been prepared[9] in order to investigate the substrate specificity of the enzyme. Preliminary results suggest that the enzyme is non-specific with respect to the position or nature of alkyl substituents on the phenyl ring. It will, however, hydrolyze only those analogs in which an unsubstituted γ-glutamyl residue is present as the acyl moiety.

A high degree of generic localization of this system is indicated from the results of tissue distribution studies. Thus far, the phenylhydrazine derivative and its hydrolase have been detected only in a group of basidiomycetes belonging to the genus *Agaricus*.[10]

During the later phases of this work, Drs. J. W. Hinman and E. G. Daniels of the Upjohn Company succeeded in isolating agaritine by modification of procedures furnished by the author. He is indebted to these workers for providing him with samples of high purity, and for valuable suggestions concerning certain aspects of the problem. Acknowledgment is also expressed to Dr. A. H. Smith, Curator of Fungi at the University of Michigan, for aid in the collection and classification of basidiomycetes. This investigation was supported in part by Grant E-2966 from the National Institute of Allergy and Infectious Diseases, United States Public Health Service.

(9) By modification of a procedure described by H. L. Yale, K. Losee, J. Martins, M. Holsing, F. M. Perry and J. Bernstein, *J. Am. Chem. Soc.*, **75**, 1933 (1953), for the synthesis of acid hydrazides.

(10) Agaritine (or substances of closely-related structure) has been detected in fruiting bodies from the following species: *campestris, comptulis, crocodilinus, diminutivus, edulis, micromegathus* and *perrarus*.

(11) Senior Research Fellow of the United States Public Health Service.

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## RADICAL REARRANGEMENTS IN BROMOALKYL RADICALS

Sir:

Rearrangements of carbonium ion systems are much faster than rearrangements in the same radical systems. For example, neopentyl, neophyl and isobutyl systems undergo complete rearrangement of the carbon skeletons in any sequence which involves a carbonium ion intermediate, but often survive conversions through the radical with little or no rearrangement. Radical-chain chlorination of neopentane yields neopentyl chloride[1] unaccompanied by rearrangements.

We wish here to report a very rapid rearrangement in radical systems. Radical chain chlorinations of (a) *i*-propyl and *n*-propyl bromides yield a common product, 1-bromo-2-chloropropane (I),

(1) F. C. Whitmore and G. H. Fleming, *J. Am. Chem. Soc.*, **55**, 4161 (1933).

and (b) *i*-butyl bromide and *t*-butyl bromide are converted to 1-bromo-2-chloro-2-methylpropane (II).
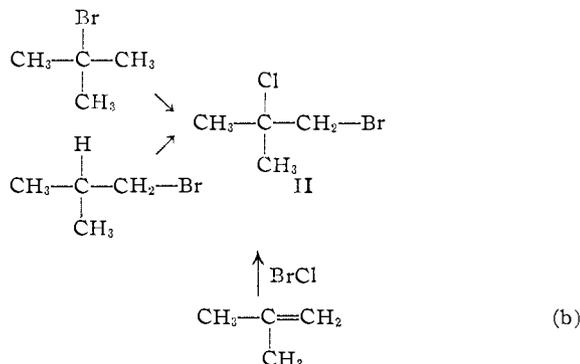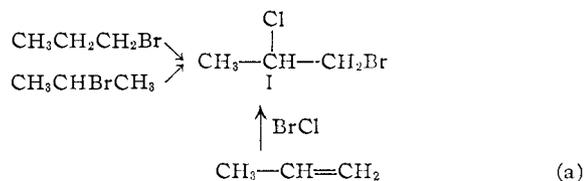
The radical-chain chlorinations were carried out at $-78°$ with *t*-butyl hypochlorite, employing photo-initiation. The products were isolated by distillation and vapor phase chromatography, capillary v.p.c. and infrared spectra being employed to demonstrate congruity of products.

Chlorination of *t*-butyl bromide gave a single product (II) in 92% yield. The structure of (II) followed from the base dehydrohalogenation of crude (II) which converted it to $(CH_3)_2C{=}CHBr$, uncontaminated by $(CH_3)_2C{=}CHCl$.

Addition of BrCl to isobutylene also produced (II). Chlorination of *i*-butyl bromide produced (II) (57%), 1-bromo-1-chloro-2-methylpropane (20%), the remainder consisting of higher boiling products.

Chlorination of *i*-propyl bromide produced mainly 2-bromo-2-chloropropane (60%) and smaller quantities (15%) of (I). The latter had infrared absorption bands identical with those of the known 1-bromo-2-chloropropane,[2] and it was not contaminated by 1-chloro-2-bromopropane.[2]

The *n*-propyl bromide chlorination produced (I) in 40% yield along with the other expected normal chlorination products.

These experiments indicate *100% rearrangement* results from removal of a hydrogen atom from the methyl groups of *i*-propyl and *t*-butyl bromides.
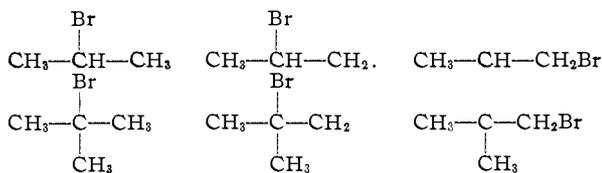
These rearrangements occur much more rapidly than the transfer of a chlorine atom to these radicals. Bromine atom migration is much faster than methyl migration.

(2) P. B. D. de La Mare and S. Galandauer, *J. Chem. Soc.*, **36** (1958).