

of water and appear 18 units lower; and $R_3 + 43$, a rearrangement peak containing one of the $-C^*H_2$ -groups. There are, of course, many more peaks in the otherwise complex spectrum which further aid in the interpretation. No appreciable peak is observed at M (mol. wt.) but one at $M + 1$,⁵ arising from an ion-molecule collision; its intensity relative to others, therefore, changes with pressure and focusing conditions and is thus easily recognized. From this the sum of $R_1 + R_2 + R_3$ can be calculated.

The proposed structures of these fragments were substantiated by comparison of the mass spectra of the reduction products of N-acetyl-gly-phe-OEt and N-acetyl-leu-ala-pro-OEt with $LiAlH_4$ and $LiAlD_4$, respectively. The spectra of the two pairs showed the expected shift in mass numbers (each $-C^*H_2$ in I becomes $-CD_2$).

This method, if applied to partial hydrolysates of natural peptides, should be very advantageous in the elucidation of their structure owing to the inherent speed and sensitivity of mass spectrometric analysis.⁶ At present we are extending our method to peptides of higher molecular weight and of other amino acids.

(5) This has been observed also with some other types of compounds; e.g., aliphatic ethers (F. W. McLafferty, *Anal. Chem.*, **29**, 1782 (1957), and references therein).

(6) The reduction can be carried out with a fraction of one milligram and yields enough material for a good spectrum.

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LIPIDS CONTAINING MONO- AND DIMETHYLETHANOLAMINE IN A MUTANT STRAIN OF *Neurospora crassa*¹

Sir:

Previous studies have shown that a choline deficient mutant of *Neurospora crassa* (strain 47904) differs from the normal wild-type (strain 1A) by its accumulation of monomethylethanolamine,² dimethylethanolamine³ and the phosphate esters⁴ of these two amines. More recent investigations have disclosed a striking difference between the phospholipids of strain 47904 and those found in strain 1A.

The ether and alcohol extractable lipids from mycelia of strains 1A and 47904 were washed free of non-lipid contaminants by the method of Folch⁵ and then subjected to prolonged acid hydrolysis. The resulting hydrolysate of the normal strain yielded choline as the predominant methylated ethanolamine. In contrast to this, the lipid hydrolysate of strain 47904 contained monomethylethanolamine, dimethylethanolamine and small

(1) This investigation was supported in part by a grant from the Division of Research Grants of the National Institutes of Health, Public Health Service RG-5794, and by the Cancer Research Funds of the University of California.

(2) N. H. Horowitz, *J. Biol. Chem.*, **162**, 413 (1946).

(3) B. Wolf and J. F. Nyc, *Biochim. et Biophys. Acta*, **31**, 208 (1959).

(4) B. Wolf and J. F. Nyc, *J. Biol. Chem.*, **234**, in press (1959).

(5) J. Folch, I. Ascoli, M. Lees, J. A. Meath and F. N. Le Baron, *ibid.*, **191**, 833 (1951).

amounts of choline (Table I). The methods used for the isolation and estimation of these amines have been described.³

TABLE I

Amine isolated after lipid hydrolysis	Strain 47904	Strain 1A
Monomethylethanolamine	13-16 ^a	...
Dimethylethanolamine	2-3	Trace
Choline	0.3-0.9	11-14

^a Data expressed as micromoles per gram of dry tissue.

Chromatographic separation of the total lipids prior to hydrolysis was carried out by the use of silicic acid columns according to the method described by Mead and Fillerup.⁶ Almost all of the lipid-bound methylated ethanolamines present in the two strains under investigation were found in the phospholipid fraction. Preliminary investigations suggest that in the choline deficient strain the phosphatidyl derivatives of monomethylethanolamine and dimethylethanolamine have replaced most of the lecithin normally found in *Neurospora crassa*. The chemical characterization of these unusual phospholipids is now in progress.

(6) J. F. Mead and D. L. Fillerup, *ibid.*, **227**, 1009 (1957).

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THE DEPHOSPHORYLATION OF CASEIN BY ALKALIES¹

Sir:

The readiness with which phosphate is cleaved by alkali from phosphoproteins such as casein and vitellin has long puzzled protein chemists. There is a considerable body of evidence that the phosphate is attached to the serine residues of these proteins by ester linkages,² yet phosphate esters are characteristically resistant to hydrolysis by alkali.³ Some years ago, Mecham and Olcott⁴ published evidence which suggests that the reaction is one of β -elimination rather than hydrolysis, but their results do not seem to have attracted wide attention. We have sought further evidence bearing on the β -elimination hypothesis.

Bovine casein was dephosphorylated with alkalis in water enriched with O^{18} , and the inorganic phosphate formed was isolated as $MgNH_4PO_4$ and converted to KH_2PO_4 . The KH_2PO_4 was analyzed for O^{18} by pyrolysis to KPO_3 and water, equilibration of the water with carbon dioxide, and analysis of the carbon dioxide in a mass spectrometer. An inspection of the results (Table I) shows that there

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This research was supported by a grant from the Carnation Co.

(2) Gertrude E. Perlmann, *Advances in Protein Chem.*, **10**, 1 (1955). The question as to whether phosphodiester and pyrophosphate-(diester) linkages occur in casein, in addition to the phosphomonoester groups, is still moot, but is not relevant here.

(3) Phosphodiesters of vicinal glycols (not possible in proteins) and phosphotriesters are exceptions to this rule, but they give monoesters and diesters, respectively, not inorganic phosphate.

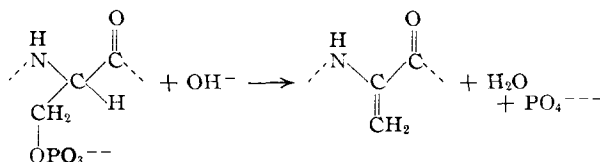
(4) D. K. Mecham and H. S. Olcott, *THIS JOURNAL*, **71**, 3670 (1949).

was essentially no incorporation of isotope into the inorganic phosphate.

TABLE I

	Dephosphorylation with Potassium hydroxide	Barium hydroxide
Atom per cent. excess O ¹⁸ in water	4.3	4.6
Casein concentration, % w./v.	5	15
Alkali concentration, <i>N</i>	0.32	0.50
Time of incubation, at 37°, hr.	15	12
Dephosphorylation, %	100	85
Atom per cent. excess O ¹⁸ in phosphate oxygen	0.04	0.01

The β -elimination of phosphate from a serine residue in a protein would be formulated as



Clearly, isotopic oxygen from the medium would not be incorporated into the phosphate during this reaction. If, on the other hand, the reaction were one of hydrolysis, and if the hydrolysis proceeded by P-O bond fission, the isolated phosphate would contain one-fourth as much O¹⁸ as the water of the medium. The hydrolysis of phosphate esters in alkali has not been studied as thoroughly as might be desirable. However, it has been demonstrated that alkali cleavage is at the P-O bond in trimethyl phosphate,⁵ in the cyclic phosphodiester of nucleosides,⁶ and in methyl α/β -D-glucopyranoside 3-phosphate and 1,2-O-isopropylidene-D-glucopyranose 3-phosphate.⁷ Furthermore, the enzymatic hydrolysis⁸ and the lanthanum hydroxide-promoted hydrolysis⁹ of phosphomonoesters, as well as the cleavage of the monoanions of these substances by water,⁹ all take place with P-O fission. Hydrolyses of phosphate esters at C-O bonds have so far been observed only in neutral or acid media. The present experiments thus support the hypothesis that the removal of phosphate from phosphoproteins by mild alkali proceeds by β -elimination. Further experiments to test the hypothesis are in progress.

Our results also would seem to rule out the existence of appreciable numbers of N-P bonds² in casein. It is difficult to write a plausible mechanism for the cleavage of such bonds which would not lead to the incorporation of oxygen from water into the phosphate.

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(5) E. Blumenthal and J. B. M. Herbert, *Trans. Faraday Soc.*, **41**, 611 (1945).

(6) D. Lipkin, P. T. Talbert and M. Cohn, *THIS JOURNAL*, **76**, 2871 (1954).

(7) E. E. Percival and E. G. V. Percival, *J. Chem. Soc.*, 874 (1945).

(8) M. Cohn, *J. Biol. Chem.*, **180**, 771 (1949); S. S. Stein and D. E. Koshland, Jr., *Arch. Biochem. Biophys.*, **39**, 229 (1952).

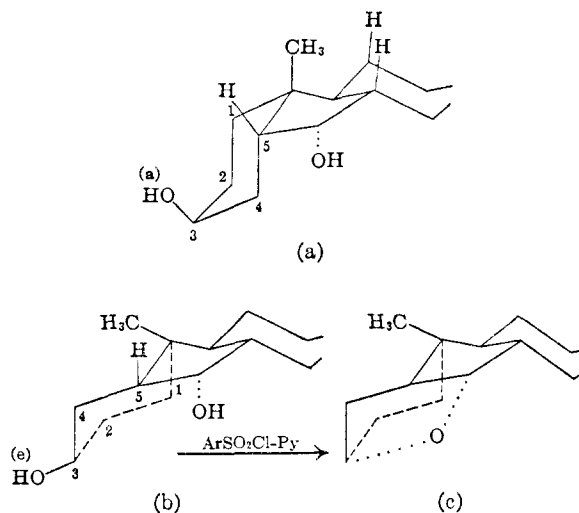
(9) W. W. Butcher and F. H. Westheimer, *THIS JOURNAL*, **77**, 2420 (1955).

B-NORCOPROSTANE DERIVATIVES

Sir:

One of us¹ established that the Butenandt ketone is a B-norstane-3,6-dione and tentatively assigned the 5β -configuration to this substance and to the parent B-norstane. Dauben and Fonken² obtained the same hydrocarbon from a B-norstanol³ derived from B-norcholesterol³ and inferred that the 3β -hydroxyl group of the stanol is equatorial because lithium aluminum hydride reduction of the 3-ketone (unhindered) afforded the original B-norstanol. They assumed that the 5β -stanol would have the conformation shown in (a) (for a 3,6-diol), in which the 3β -hydroxyl group is axial, and concluded that the B-norstanol belongs to the 5α -series, in which the hydroxyl group would be equatorial. On the other hand, the rotatory dispersion curve indicates that the ketone probably is B-norcopropane-3-one.⁴

In seeking to reconcile the seemingly conflicting evidence, we noticed that ring A of form (a) can undergo a flip to produce the conformation (b) in



which the 3β -hydroxyl is equatorial. Since this flip tilts the methyl group away from ring B and so relieves a severe 10-CH₃:11 β -H interaction (and a lesser 10-CH₃:8 β -H interaction), (b) should be more stable than (a). We have now prepared the $3\beta,6\alpha$ -diol (b) and converted it into the oxide (c) and so proved that the diol and the related stanol are in fact B-norcopropane derivatives. Sodium borohydride reduction of B-norcopropane-3,6-dione gave diol (b), m.p. 140-141°, [α]_D -2° and an isomeric diol, m.p. 170-172° [α]_D +35° (found C, 79.91; H, 11.95, and C, 80.04; H, 11.97), separated by chromatography of the diacetates; acetate (b), an oil, and the isomeric diacetate, 93-94°, [α]_D +38° (C, 75.77; H, 10.60). Both diols on oxidation gave the original dione. Heated in pyridine with benzenesulfonyl chloride, diol (b) afforded in 40% yield the oxide (c), m.p. 85.5-86.5°, [α]_D +31° (C, 83.64; H, 11.85; mol

(1) L. F. Fieser, *THIS JOURNAL*, **75**, 4386 (1953).

(2) W. G. Dauben and G. J. Fonken, *ibid.*, **78**, 4736 (1956).

(3) F. Šorm and H. Dykova, *Coll. Czech.*, **13**, 407 (1948).

(4) C. Djerassi, D. Marshall and T. Nakano, *THIS JOURNAL*, **80**, 4853 (1958).