	Crystals	M.p., °C.	C	H	P		Mol. wt.
$(C_6H_5)_3PCr(CO)_5$	Pale yellow	127–128	60.8	3.3	6.8	Cr, 11.3	458
$(C_6H_5)_3PMo(CO)_5$	White	138–139	55.6	3.0	5.7	Mo, 19.5	495
$(C_6H_5)_3PW(CO)_5$	Pale yellow	146–147	47.0	2.7	5.0	W, 31.5	593
$(NCH_2CH_2)_3PCr(CO)_5$	White	136–137	43.8	3.0	7.7	Cr, 13.3	N, 10.9
$(C_6H_5O)_3PCr(CO)_5$	White	59.5–60	55.0	3.1	6.2	Cr, 10.2	495
$((C_6H_5O)_3P)_2Cr(CO)_4$	White	148–149	60.8	4.0	7.8	Cr, 6.6	...
$((C_6H_5O)_3P)_3Cr(CO)_3$	White	126–126.5	64.4	4.6	8.2	Cr, 5.0	...
$((C_4H_9O)_3P)_2Cr(CO)_4$	Pale green liquid	230 b.p. (1 mm.)	51.3	9.2	9.4	Cr, 8.2	660
$(C_6H_5)_3AsCr(CO)_5$	Yellow	135–135.5	55.4	3.0	...	Cr, 9.3	As, 14.9
$(C_6H_5)_3SbCr(CO)_5$	Yellow	147–149	50.6	2.7	...	Cr, 9.3	...

pyridine[5] and *o*-phenylenebis-(dimethylarsine)[6] are known to yield substituted metal carbonyl compounds.

Triphenylphosphine derivatives of zerovalent Group VIB metal compounds have been prepared, however, from substituted carbonyls such as monoammine chromium pentacarbonyl or pyridine-chromium pentacarbonyl, yielding bis-(triphenylphosphine)-chromium tetracarbonyl,[7] and from compounds such as cycloheptatriene-molybdenum tricarbonyl, yielding tris-(triphenylphosphine)-molybdenum tricarbonyl.[8]

We have found that stable, non-volatile, monomeric compounds, usually crystalline, are formed in high yield by treating a trivalent phosphorus compound with a metal hexacarbonyl or arene-metal tricarbonyl of a Group VIB metal in a solvent such as diglyme, bis(2-methoxyethyl) ether, under reflux. Filtration of the reaction mixture gave clear, colored filtrates from which the products were obtained by removal of excess solvent. They were purified by repeated crystallization from a mixture of chloroform and ethanol. The compounds were characterized by elemental analyses, molecular weight determinations when possible, and by infrared spectra (characteristic bands in the 5 μ region).

The analytical results showed that either one or two of the carbon monoxide groups of metal hexacarbonyls were displaced readily by ligands such as triphenylphosphine, triphenyl phosphite, tris-(2-cyanoethyl)-phosphine and tri-*n*-butyl phosphite, *e.g.*

$$Cr(CO)_6 + 2P(OC_6H_5)_3 \longrightarrow [P(OC_6H_5)_3]_2Cr[CO]_4 + 2CO$$

With arene-chromium tricarbonyls, such as benzene-chromium tricarbonyl or durene-chromium tricarbonyl, displacement of the aromatic ring by three triphenylphosphite molecules occurred, *e.g.*

$$(C_6H_6)Cr(CO)_3 + 3P(OC_6H_5)_3 \longrightarrow [P(OC_6H_5)_3]_3Cr[CO]_3 + C_6H_6$$

Wirh trivalent compounds of other Group VA elements, such as triphenylarsine and triphenylstibine, similar reactions occurred.

Some representative compounds are tabulated.

The preparation of further compounds derived from zerovalent derivatives of the Group VIB metals and trivalent compounds of Group VA elements is now in progress. Experimental details concerning the preparation and reactions of such compounds will be published later.

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(5) W. Hieber and F. Mühlbauer, *Z. anorg. allgem. Chem.*, **221**, 341 (1935).

(6) H. L. Nigam and R. S. Nyholm, *Proc. Chem. Soc.*, 321 (1957).

(7) H. Behrens and W. Klek, *Z. anorg. allgem. Chem.*, **292**, 151 (1957).

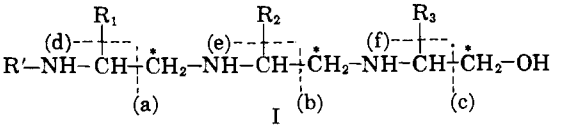
(8) E. W. Abel, M. A. Bennett, R. Burton and G. Wilkinson, *J. Chem. Soc.*, 4559 (1958).

APPLICATION OF MASS SPECTROMETRY TO STRUCTURE PROBLEMS. I. AMINO ACID SEQUENCE IN PEPTIDES

Sir:

We have investigated the mass spectra of polyamino alcohols, obtainable[1] by reduction of small peptides with $LiAlH_4$, because fragments due to rupture of the carbon–carbon bond alpha to the amino groups should yield valuable information about the structure of the parent peptide. Another reason for the choice of the polyamines was their greater volatility compared with the corresponding peptide, an important factor in mass spectrometry.

The spectra of all the polyamino alcohols determined thus far,[2] in fact, exhibit a characteristic pattern due to preferential rupture of the bonds indicated below for a triamino alcohol[3] of molecular weight M:



I

(R′ = H or $CH_3\overset{*}{C}H_2$–; $-\overset{*}{C}H_2-$ corresponds to –CO– in the peptide.)

Cleavage at (a) gives rise to peaks at mass $R_1 + 28 + R'$[4] and $115 + R_2 + R_3$; at (b): $R_1 + R_2 + 70 + R'$ and $R_3 + 73$; at (c): $R_1 + R_2 + R_3 + 112 + R'$ and 31. The other important fragments [cleavage at (d), (e), and (f)] are $M - R_1$, $M - R_2$ and $M - R_3$ which may also lose the elements

(1) P. Karrer and B. J. R. Nicolaus, *Helv. Chim. Acta*, **35**, 1581 (1952).

(2) The N-acetyl ethyl esters of nine di- and three tripeptides containing gly, ala, leu, pro, phe, ser and asp were synthesized and reduced. Three free dipeptides containing also val and norval were reduced directly. The N-acetyl derivatives were used because they are easily synthesized and on reduction yield N-ethyl compounds whose mass spectra follow the same general trend except for the displacement for 28 mass units of the N-terminal fragments. The spectra were determined with a CEC 21-103C mass spectrometer, equipped with a heated inlet system operated at 140°, using an ionization potential of 70 or 11 v.

(3) The spectra of polyamino alcohols from other peptides follow an analogous pattern.

(4) Most important peak in the spectrum.

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.

(7) This investigation was supported by a research grant (RG-5472) from the National Institutes of Health, Public Health Service.

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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] dimethylethanolamine[3] and the phosphate esters[4] 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 Folch[5] 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 lipide hydrolysate of strain 47904 contained monomethylethanolamine, dimethylethanolamine and small 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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RECEIVED FEBRUARY 27, 1959

THE DEPHOSPHORYLATION OF CASEIN BY ALKALIES[1]

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 Olcott[4] 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 alkalies 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) 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).

(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).