glacial acetic acid–0.1 *N* HCl (2,2,1).[3] Material from the left band of Fig. 1 gave a good yield of a DNP-peptide identified by hydrolysis, partition ratios and chromatography as DNP-Val-Leu. That from the right gave none of this material. From the work of Rhinesmith, Schroeder and Martin,[2] it appears that the left band of Fig. 1 contains protein corresponding to their α chain while the right, lacking this terminal sequence, probably contains the terminal Val-His-Leu sequence of their β chain.

The analytical data in the table show wide differences for most of the amino acid residues.

The globins from horse hemoglobin recently have been resolved.[6]

The over-all results reported in this communication are interesting in connection with those reported by Singer and Itano.[7]

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RECEIVED MARCH 19, 1957

OXIDATIVE METABOLISM OF ESTROGENS[1]

Sir:

The major path of metabolism of the estrogenic hormones in man is oxidative. Estrone and estradiol are interconvertible *in vivo* and this study was initiated to determine whether estrone or estradiol served as the immediate precursor of the more oxygenated metabolites. A mixture of estradiol 6,7-H^3 and estrone-16-C^{14} was injected rapidly and intravenously in human subjects. Urine collections were obtained at frequent intervals and a blood sample was obtained at 30 minutes after injection. Estrone, estradiol-17β, estriol (16α,17β), epiestriol (16β,17β) and 2-methoxyestrone were isolated and purified to radiochemical homogeneity. These were analyzed for C^{14} and H^3 and the ratio (C^{14}/H^3) of the two isotopes[2] measured. Three studies with concordant results were made on different patients using differing weight ratios of estrone and estradiol. A representative experiment is shown in Table I.

The values obtained show that estrone approaches C^{14}/H^3 of the injected mixture more rapidly than does estradiol. Earlier in the experiment when these values for estrone and estradiol are different, the ratios for estriol, epiestriol and 2-methoxyestrone agree with the estrone ratio. The

(1) We express our appreciation to our colleagues Drs. Leon Hellman and Barnett Zumoff, who made possible the studies with the patients, and for the support of grants from the American Cancer Society and from the National Cancer Institute (CY-3207), United States Public Health Service.

(2) The ratio is obtained from the counts per minute measured for C^{14} and H^3 in a Packard "Tri-Carb" Scintillation Spectrometer, Model 314, and is therefore an arbitrary value. Portions of the injection solution were counted concurrently with the metabolites to insure that standard conditions were observed.

TABLE I

ISOTOPE RATIO OF URINARY AND BLOOD METABOLITES

Dose: Estrone-16-C^{14} 10.9 mg., 29.5 μc. Estradiol-6,7-H^3 1.5 mg., 150 μc. Measured isotope ratio,[2] C^{14}/H^3 = 0.92. *Insufficient metabolite for analysis.

Time of urine collection, minutes	C^{14}/H^3				
	Estrone	Estradiol	Estriol	Epi-estriol	2-Methoxy-estrone
0–30	1.15	0.08	*	*	*
30–60	0.84	.39	0.72	*	0.82
60–120	.81	.62	.77	0.77	.82
120–180	.83	.66	.81	.76	.87
240–300	.85	.74	.80		.91
360–540	.87	.81	.86		.85
Blood sample at 30 minutes					
"Free"	4.00	0.04	*		
"Conjugated"	0.85	0.48	*		

free and conjugated blood steroids show different C^{14}/H^3; the conjugates mirrored the urinary values found from 30 to 60 minutes later.

It can be concluded that (1) estrone serves as the principal, if not the exclusive, substrate for hydroxylation at C-16 to give both the α and β hydroxy compounds. (2) Estrone similarly serves as the principal substrate for hydroxylation in ring A. (3) The combined rate of all reactions in the body by which estradiol is oxidized to estrone is greater than the similar processes reducing estrone to estradiol. (4) Virtually only estrone is available for metabolic transformation, *i.e.*, conclusions 1 and 2 may well be a consequence of conclusion 3. (5) While estradiol may be the hormone produced by the ovaries, peripheral hormone action may be effected largely through estrone, which formally is a metabolite.

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REACTION PRODUCTS OF GROUP VIB METAL CARBONYL COMPOUNDS WITH ORGANIC COMPOUNDS OF TRIVALENT GROUP VA ELEMENTS

Sir:

We wish to report the preparation of compounds formed by the reaction of trivalent Group VA compounds (such as tertiary phosphines and phosphites) with carbonyl compounds of Group VIB metals (such as chromium hexacarbonyl and benzene chromium tricarbonyl).

The reactions of metal carbonyls with trivalent phosphorus compounds to give derivatives of carbonyls of the first series of the Group VIII metals (*e.g.*, bis-(triphenylphosphine)-nickel dicarbonyl)[1] and of Group VII metals (*e.g.*, triphenylphosphine-manganese tetracarbonyl)[2] have previously been described. No reactions between the hexacarbonyls of the Group VIB metals and trivalent phosphorus compounds have been reported, although reactions of the hexacarbonyls with ammonia,[3,4]

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(3) W. Hieber and W. Aheck, *ibid.*, **7b**, 320 (1952).

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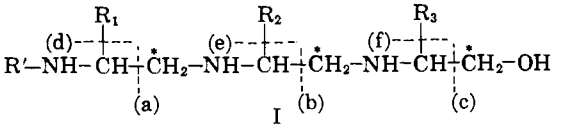
APPLICATION OF MASS SPECTROMETRY TO STRUCTURE PROBLEMS. I. AMINO ACID SEQUENCE IN PEPTIDES

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.