three products: androst-4-ene-3,17-dione, testosterone and, surprisingly, *testosterone acetate*. In a typical preparative experiment 3.0 g. of progesterone, after 48-hour incubation with a 72-hour growth of *C. resinae*, afforded 0.31 g. of testosterone acetate, m.p. 138.5–140°, $[\alpha]_D + 85°$ (*c*, 0.81 in acetone) (reported[6] m.p. 138°, $[\alpha]_D + 87.5°$ (ethanol)), whose infrared spectrum was identical to that of an authentic specimen.

The testosterone acetate could arise either as an intermediate in the cleavage path, or as an acylation product of testosterone, although microbiological acylation of steroids has not been reported. To distinguish between these two possibilities, testosterone was incubated with *C. resinae* in the same manner as the progesterone had been; under these circumstances no testosterone acetate was found but only androst-4-ene-3,17-dione was obtained. Since it is conceivable that the acetylation of testosterone could be influenced by the presence of intermediary metabolites, the source of the acetate had to be established in order to clarify the reaction sequence. That the acetate was indeed derived solely from the progesterone side-chain was shown by incubation of progesterone-21-C^{14} with *C. resinae*. In Experiment I, in which a spore inoculum was used, some residual progesterone was recovered, which permitted a check of the quantitative procedure employed. In Experiment II, in which a vegetative inoculum was used, essentially no progesterone remained.

The testosterone acetate and the residual progesterone were isolated and purified by paper chromatography (CM System).[7] After elution of the appropriate portions of the paper chromatograms, the yields of these compounds were determined by measurement of the light absorption at 242 $m\mu$.[7] Radioactivities were determined in a Packard Tri-Carb Scintillation Spectrometer[8] and from these values the specific radioactivities were calculated. The results, in each case the average of duplicate determinations, are shown in the following table.

	Experiment I		Experiment II	
	Yield, %[9]	Specific activity[10]	Yield, %[9]	Specific activity[10]
Progesterone (Substrate)	...	8649	...	8649
Progesterone (Recovered)	17.5	8460	0	...
Testosterone Acetate	17.5	8626	18.2	9320

It seems clear that, at least for *C. resinae*, cleavage of the progesterone side-chain involves testosterone acetate as part of the degradation path. To our knowledge this is the first demonstration that this biological oxidative cleavage proceeds by a pathway similar to that of the nonenzymatic action of peracids on ketones, as suggested by Fried, *et al.*[2] It is possible that other microörganisms degrade progesterone by this same pathway but that the acetate intermediate has not been detected because of the high level of esterase present in those cases. The relationship of this metabolic path to mammalian degradation of similar molecules remains to be considered.

RESEARCH LABORATORIES GUNTHER S. FONKEN
TH UPJOHN COMPANY HERBERT C. MURRAY
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RECEIVED JULY 29, 1960

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(5) *Cladosporium resinae* (Lindau) de Vries f. *avellaneum* de Vries. Our strain has been deposited with the Centralbureau voor Schimmelcultures, Baarn, Netherlands.

(6) A. Butenandt and G. Hanisch, *Z. physiol. Chem.*, **237**, 89 (1935).

(7) L. M. Reineke, *Anal. Chem.*, **28**, 1853 (1956).

(8) We are indebted to Dr. R. C. Thomas, Department of Physical and Analytical Chemistry, The Upjohn Co., for the radioactivity determinations.

(9) Based on substrate added.

(10) Disintegrations per minute per micromole.

HEAT OF FORMATION OF THE MOST STABLE FORM OF METABORIC ACID, HBO_2(c,I)

Sir:

A value has not been reported for the heat of formation of the most stable crystalline form of metaboric acid, the cubic HBO_2(c,I), which is of interest as one of the possible products of combustion of boron-containing fuels. We have derived the heat of formation by measurement of the heat of reaction of HBO_2(c,I) and of H_3BO_3(c) with aqueous sodium hydroxide.

Although HBO_2(c,I) has been prepared previously,[1] some workers have had difficulty in obtaining sizable quantities; we have prepared it by two methods. In the simplest, recrystallized orthoboric acid was air-dried at room temperature and then dehydrated in loosely glass-stoppered reagent bottles at 120 to 130° for 4 or 5 weeks. Most of the resulting crystals were approximately 1 mm. in size. A disadvantage of the method is that the product may contain a large proportion of the monoclinic form, HBO_2(c,II); this depends on the extent to which the stopper limits the escape of gases from the container. The second method produced crystals generally of about 0.5 mm. in size; large crystals occasionally were formed. In this method, a mixture of a few seed crystals of HBO_2(c,I), 5 g. of recrystallized H_3BO_3, and 15 g. of the orthorhombic HBO_2(c,III), was placed in 100-ml. glass ampoules. Air was removed from the ampoule by pumping for a few minutes with a mechanical pump, and the ampoule then was sealed. After heating for 2 to 7 days at 180°, crystallization of the melt was visible; 2 to 5 weeks was allowed for crystal aggregation. The crystals were washed briefly with distilled water and then with methanol, which, unlike water, did not etch the surface of the crystals upon long exposure.

The product was identified by X-ray diffraction, refractive index, and density measurements. When weighed samples of the HBO_2(c,I) were dissolved in boiling water and the solutions were titrated with sodium hydroxide solution in the presence of D-mannitol, 99.9% of the theoretical amount of boric acid was found. It was also possible to make a quick identification, and at least a partial separation, of the three crystalline metaboric acids and orthoboric acid by flotation in these liquids: spectroscopic (H_2O-free) carbon tetrachloride, den-

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(1) F. C. Kracek, G. W. Morey, and H. E. Merwin, *Am. J. Sci.*, **35A**, 143 (1938).

sity = 1.60 g./ml.; ethyl iodide, density = 1.92 g./ml.; and ethylene bromide, density = 2.17 g./ml.

In order to achieve complete solution of the HBO_2(c,I) in 20 to 30 minutes for calorimetric experiments, it was necessary to crush the sample to pass a No. 200 standard sieve and use 2*N* sodium hydroxide solution at 40° as the calorimetric solution. The calorimetric samples were placed in spherical glass ampoules and heated at 100° for about 30 minutes to drive off adsorbed moisture. The ampoules then were sealed under vacuum and weighed.

The solution calorimeter consisted of a vacuum-jacketed, Pyrex-glass vessel having a capacity of about 500 ml. of solution. A metal cap supported a platinum resistance thermometer, a glass stirrer, a precision immersion heater used in electrical calibrations, and a glass sample-holder. Electrical calibrations were made on the initial systems.

In four experiments, samples of HBO_2(c,I), 0.3 to 0.4 g. (prepared by the second method described above), reacted with 2 *N* sodium hydroxide at 40°; the corrected temperature rise was approximately 0.1° and the mean heat of reaction, $\Delta H(40^\circ) = -27.44 \pm 0.042$ kj./mole. Similarly, four experiments with 1.5 to 1.8 g. of H_3BO_3(c) produced a temperature rise of a little more than 0.2° and $\Delta H(40^\circ) = -21.46 \pm 0.02$ kj./mole. The uncertainties are twice the standard deviation of the mean.

The difference in the heats of reaction at 40° is -5.98 ± 0.05 kj./mole or -1.429 ± 0.011 kcal./mole. We estimate a thermal coefficient, $\Delta C_p \sim -10$ cal./deg. mole, which gives -1.28 kcal./mole for the difference in the heats of reaction at 25°. By using $\Delta Hf^\circ = -68.317$ kcal./mole for the heat of formation of liquid water,[2] and $\Delta Hf^\circ = -262.16$ kcal./mole for H_3BO_3(c),[3] we obtain $\Delta Hf^\circ_{(298.15)} = -192.56 \pm 0.33$ kcal./mole for HBO_2(c,I).

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(2) F. D. Rossini, D. D. Wagman, W. H. Evans, S. Levine and I. Jaffe, "Selected Values of Chemical Thermodynamic Properties," NBS Circular 500, U. S. Government Printing Office, Washington 25, D. C., 1952.

(3) W. H. Evans, E. J. Prosen, and D. D. Wagman, "Thermochemistry and Thermodynamic Functions of Some Boron Compounds," in "Thermodynamic and Transport Properties of Gases, Liquids, and Solids," Y. S. Touloukian, editor, McGraw-Hill Book Company, Inc., New York, N. Y., 1959, p. 226.

NATIONAL BUREAU OF STANDARDS MARTHADA V. KILDAY
WASHINGTON, D. C. EDWARD J. PROSEN

RECEIVED SEPTEMBER 12, 1960

A NEW CLASS OF PHOSPHORUS-NITROGEN COMPOUNDS[1]

Sir:

The literature shows no successful characterizations of chlorine free phosphorus nitrogen compounds resulting from the reaction of phosphorus trihalides and primary aliphatic amines. However, working with excess methylamine in the absence of solvent below room temperature, we have isolated a crystalline white solid in high yield and report it as the first member of a new class of phosphorus nitrogen compounds.

It has the empirical composition $P_2N_3(CH_3)_3$, m.p. 122.0–122.8°; b.p. 303–304° at 739 mm. *Anal.* Calcd.: C, 24.17; H, 6.08; N, 28.19; P, 41.56. Found: C, 23.92; H, 6.03; N, 28.19; P, 41.32.

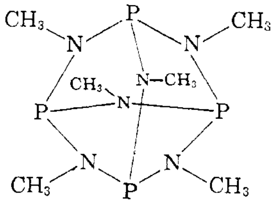
Initially it is obtained with the amine hydrochloride from which it is readily separated using dried petroleum ether and working in a nitrogen atmosphere (moisture sensitive). The phosphorus-nitrogen product is purified readily by sublimation, is soluble in a wide variety of organic solvents, initially insoluble in water but slowly dissolves over a period of a few days. No other products are obtained from the reaction, as established by elemental analysis directly on the ether soluble and the ether insoluble fractions.

Infrared measurements in Nujol mulls and in benzene solution were nearly identical showing strong absorptions with rather sharp bands at 2860 cm.$^{-1}$, 2790 cm.$^{-1}$, 1440 cm.$^{-1}$, and 1155 cm.$^{-1}$. The latter band is in the region of the P–N stretching frequency found in the lower membered ring structures in the phosphonitrilic series.[2]

Cryscopic measurements in benzene gave molecular weight results (296 ± 15) in agreement with vapor phase determinations near 200° (313 ± 10, assuming ideal gas behavior) and indicates a molecular formulation $P_4N_6(CH_3)_6$.

N.m.r. measurements on benzene solutions showed one peak, having a one-two-one spin-spin splitting pattern. The latter data indicate equivalent protons each split by two equivalent phosphorus atoms. The chemical shift relative to water, + 2.52 p.p.m., is characteristic of a N-CH_3 grouping.[3] In addition the J_{P-H} coupling constant, 16.7 ± 0.6 cps., is in the range characteristic of the P–N–C–H linkage.[4]

The above data strongly indicate a cage structure analogous to that of phosphorus trioxide, P_4O_6, containing a tetrahedral distribution of phosphorus atoms with oxygens strung along the edges, here replaced by six N–CH_3 groups. The latter structure is made even more attractive when it is realized that the N–CH_3 group is isoelectronic with the oxygen atom.



Chemical data are in agreement with the structural interpretation in that reactions might be expected to proceed toward a phosphorus pentoxide structure, P_4O_{10}, by coördinating the four phosphorus atoms with electron acceptor groups since each phosphorus has an unshared pair in the tri-

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(1) Supported in part by the Office of Ordnance Research, U. S. Army.

(2) H. J. Emeleus and A. G. Sharpe, ed., "Advances in Inorganic Chemistry and Radiochemistry," Vol. I, Academic Press Inc., New York, N. Y., 1959, p. 372.

(3) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p. 272.

(4) Pople, *et al.*, *ibid.*, p. 351.