quadricoördinate Ni(II) complexes, diamagnetic as solids, become partially paramagnetic when dissolved in non-coördinating solvents is now well documented in the case of the salicylaldimines.[5,6,7,8] A second interpretation of the phenomenon as a conformational equilibrium between planar and tetrahedral forms[5,6,7] now may be safely ruled out in view of dielectric polarization data[8] and because the magnetic and, more especially, the spectral properties of the salicylaldimine complexes in this and other work[5,6,7,8] bear no resemblance to recent observations of tetrahedral Ni(II) complexes in which the symmetry is nearly tetrahedral[9] or markedly lower.[10]

We wish to report results for certain N–R salicylaldimine complexes (R = substituent) which indicate that at least in certain instances intermolecular association is a strong contributing factor to partial paramagnetism in solution. Heretofore the magnetic behavior had been attributed to changes in intrinsic ligand field strengths upon dissolving[3] and to solvation.[4] Previous magnetic studies have been carried out in which R = alkyl,[1,2,5,6,7,8] commonly methyl.[5,6,7,8] When R = Et (I), *n*-Pr (II), *n*-Bu (III), we find in benzene and chloroform (in accord with Sacconi[8]) that the nickel is feebly paramagnetic at 300°K. and monomeric or very slightly associated in freezing benzene, but when R = *i*-Pr (IV), *sec*-Bu (V), cyclopentyl (VI), the complexes are strongly paramagnetic in solution and associated. The following results were obtained in benzene at comparable concentrations. Polymerization, however, does

	μ (B.M.)	Mol. wt. Calcd.	Found
I	0.23	355	359
II	0.29	383	384
III	0.45	411	435
IV	2.18	383	527
V	2.10	411	501
VI	2.58	435	~640

not necessarily account fully for the observed paramagnetism. Association may occur via weak Ni...O interactions, analogous to those found in Cu(II) dimethylglyoxime[11] and bis-(salicylaldehyde)-ethylenediimine Cu(II),[12] which provide a tetragonal ligand field component, thus enhancing the triplet character of the ground state.[4] Similar conclusions have been drawn concerning the magnetic properties of certain substituted Ni(II) acetylacetonates.[13]

It was postulated[14] that if spin-free planar Ni(II) complexes do exist, it might be possible to alter singlet–triplet distributions at a given temperature in complexes of the type $Ni-O_2N_2$. Accordingly, we have prepared and examined an extensive number of N-substituted salicylaldimine complexes. We have found that by altering R it is possible in certain instances to transform the nickel from a predominantly triplet to an essentially singlet state in benzene and chloroform solutions at room temperature. In chloroform when R = C_6H_5 (VII), *p*-C_6H_4F (VIII), *p*-C_6H_4Cl (IX), *p*-tolyl (X), *m*-tolyl (XI), the magnetic moments (25–28°) are: VII, 2.92; VIII, 2.93; IX, 2.91; X, 3.05; XI, 3.25 B.M. However, where R = *o*-tolyl (XII) and 1,5-$C_6H_3(CH_3)_2$ (XIII) the moments are XII, 0.95; XIII, 0.20 B.M. Similar results were obtained in benzene, *e.g.*, VII, 3.08; VIII, 3.04; X, 3.14; XI, 3.26; XII, 0.96, XIII, 0.30 B.M. The ground state is predominantly a triplet[15] in VII–XI, essentially a singlet in XII, and nearly a pure singlet in I–III, XIII. Such large variations in the moments of salicylaldimine complexes or other magnetically anomalous Ni(II) complexes have not been observed previously. The extent to which association affects these variations is being investigated.

A more detailed report of magnetic and spectral studies of these and other Ni(II) salicylaldimine complexes will be the substance of a later communication.

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(15) Orbital contributions in spin-free planar monomeric Ni(II) complexes are not known but may be estimated as 0.3–0.6 B.M.

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(5) B. Willis and D. P. Mellor, THIS JOURNAL, **69**, 1237 (1947).

(6) S. Fujii and M. Sumitani, *Sci. Repts. Tohoku University*, 1st Ser., **37**, 49 (1953).

(7) H. C. Clark and A. L. Odell, *J. Chem. Soc.*, 3431 (1955).

(8) L. Sacconi, P. Paoletti and G. DelRe, THIS JOURNAL, **79**, 4092 (1957).

(9) D. M. Gruen and R. L. McBeth, *J. Phys. Chem.*, **63**, 393 (1959); N. S. Gill and R. S. Nyholm, *J. Chem. Soc.*, 3997 (1959).

(10) F. A. Cotton and D. M. L. Goodgame, THIS JOURNAL, in press.

(11) E. Frasson, R. Bardi and S. Bezzi, *Acta Cryst.*, **12**, 201 (1959).

(12) D. Hall and T. N. Waters, *J. Chem. Soc.*, 2644 (1960).

(13) J. P. Fackler, Jr., and F. A. Cotton, THIS JOURNAL, **82**, 5005 (1960).

(14) R. H. Holm, *Ibid.*, **82**, Nov. 5 (1960).

PATHWAY OF PROGESTERONE OXIDATION BY CLADOSPORIUM RESINAE

Sir:

The ability of various microörganisms to cleave oxidatively the side-chain of C_{21} steroids to give C_{19} steroids having a 17-ketone, 17β-alcohol, or a ring D lactone function is well-known.[1] A reasonable path for this type of oxidation of progesterone would appear to be: progesterone → testosterone → androst-4-ene-3,17-dione → testololactone.[2] More recently Sebek, *et al.*,[3] have suggested that *Penicillium lilacinum* Thom degrades progesterone by the path: progesterone → 20β-hydroxypregn-4-en-3-one → testosterone → androst-4-ene-3,17-dione → testololactone.

We have found that incubation of progesterone aerobically[4] with *Cladosporium resinae*[5] affords

(1) D. H. Peterson, "Fourth International Congress of Biochemistry. Vol. IV. Biochemistry of Steroids," Pergamon Press, New York, N. Y., 1959, p. 93 f.; S. H. Eppstein, P. D. Meister, H. C. Murray, and D. H. Peterson, "Vitamins and Hormones," Academic Press Inc., New York, N. Y., 1956, Vol. XIV, p. 392 f.; E. Vischer and A. Wettstein, "Advances in Enzymology," Vol. XX, Interscience Publishers, Inc., New York, N. Y., 1958, p. 237 f.

(2) J. Fried, R. W. Thoma and A. Klingsberg, THIS JOURNAL, **75**, 5764 (1953).

(3) O. K. Sebek, L. M. Reineke, H. C. Murray, and D. H. Peterson, unpublished results, cited in ref. 1a, p. 93.

(4) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh, THIS JOURNAL, **74**, 5933 (1952).

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 mμ.[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.

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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-

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