

quadracoördinate 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 data⁸ and because the magnetic and, more especially, the spectral properties of the salicylalimine 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 tetrahedral⁹ or markedly lower.¹⁰

We wish to report results for certain N-R salicylalimine 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 dissolving³ 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 Sacconi⁸) 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) dimethylglyoxime¹¹ and bis-(salicylaldehyde)-ethylenedimine 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 postulated¹⁴ that if spin-free planar Ni(II) complexes do exist, it might be possible to alter singlet-triplet distributions at a given tem-

(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).

perature in complexes of the type Ni-O₂N₂. Accordingly, we have prepared and examined an extensive number of N-substituted salicylalimine 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 = C₆H₅ (VII), *p*-C₆H₄F (VIII), *p*-C₆H₄Cl (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-C₆H₃(CH₃)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 triplet¹⁵ in VII–XI, essentially a singlet in XII, and nearly a pure singlet in I–III, XIII. Such large variations in the moments of salicylalimine 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) salicylalimine complexes will be the substance of a later communication.

Financial support under grant NSF-G12312 was provided by the National Science Foundation to whom grateful acknowledgment is made.

(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.

DEPARTMENT OF CHEMISTRY
HARVARD UNIVERSITY
CAMBRIDGE 38, MASSACHUSETTS

R. H. HOLM
T. M. MCKINNEY

RECEIVED AUGUST 29, 1960

PATHWAY OF PROGESTERONE OXIDATION BY CLADOSPORIUM RESINAE

Sir:

The ability of various microorganisms to cleave oxidatively the side-chain of C₂₁ steroids to give C₁₉ steroids having a 17-ketone, 17 β -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 \rightarrow testosterone \rightarrow androst-4-ene-3,17-dione \rightarrow testolactone.² More recently Sebek, *et al.*,³ have suggested that *Penicillium lilacinum* Thom degrades progesterone by the path: progesterone \rightarrow 20 β -hydroxypregn-4-en-3-one \rightarrow testosterone \rightarrow androst-4-ene-3,17-dione \rightarrow testolactone.

We have found that incubation of progesterone aerobically⁴ with *Cladosporium resiniae*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°, $[\alpha]_D + 85^\circ$ (c, 0.81 in acetone) (reported⁶ m.p. 138°, $[\alpha]_D + 87.5^\circ$ (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 acylation 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-C¹⁴ 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 μ .⁷ Radioactivities were determined in a Packard Tri-Carb Scintillation Spectrometer⁸ 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

(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.

proceeds by a pathway similar to that of the non-enzymatic action of peracids on ketones, as suggested by Fried, *et al.*² It is possible that other microorganisms 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
THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN

GUNTHER S. FONKEN
HERBERT C. MURRAY
LESTER M. REINEKE

RECEIVED JULY 29, 1960

HEAT OF FORMATION OF THE MOST STABLE FORM OF METABORIC ACID, HBO₂(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 HBO₂(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 HBO₂(c,I) and of H₃BO₃(c) with aqueous sodium hydroxide.

Although HBO₂(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, HBO₂(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 HBO₂(c,I), 5 g. of recrystallized H₃BO₃, and 15 g. of the orthorhombic HBO₂(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 HBO₂(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 (H₂O-free) carbon tetrachloride, den-

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