was obtained. Washing of this residue once with 20 ml. of cold ethanol gave 9.0 g. (62% yield) of the desired product, m.p. 114–115° (reported[2] m.p. 116.5–118°, reported[3] 114°).

N-Alkyl Cleavage in Acid Hydrolysis of Norbornane γ-Lactams

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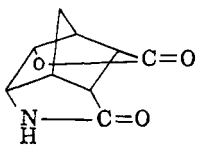
When the lactone-lactam I previously described by Worrall,[2] was refluxed in 5% hydrochloric acid, N-alkyl cleavage occurred and the product, isolated in 85% yield, was found to be identical with an authentic sample of the nortricyclenic acid lactone II.[3,4] In a similar manner III was found to give IV which was converted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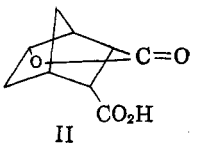
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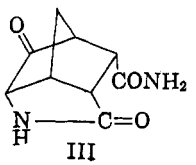
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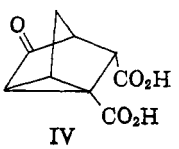
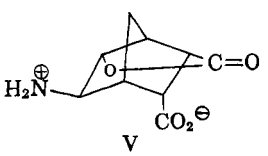

IV

into II by reduction with sodium borohydride followed by heating. The ketone IV had been previously prepared by oxidation of II.[4] The amino acid lactone V,[5] is converted into its hydrochloride under the conditions mentioned above, indicating that the N-alkyl cleavages observed occur in the lactams instead of the amino acids. The unusual N-alkyl cleavages observed are considered to proceed *via* an intermediate bridged carbonium ion such as VI. In a similar manner N-*t*-butylisobutyramide, VII, evolved isobutylene when refluxed in 20% hydrochloric acid.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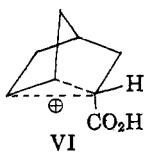
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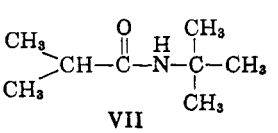
VII

(1) National Defense Education Act Fellow, 1959–1962.
(2) W. S. Worrall, *J. Am. Chem. Soc.*, **82**, 5707 (1960).
(3) K. Alder and F. Brockhagen, *Ber.*, **87**, 167 (1954).
(4) A. Winston and P. Wilder, Jr., *J. Am. Chem. Soc.*, **76**, 3045 (1954).
(5) L. H. Zalkow and C. D. Kennedy, the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September 9–14, 1962, Abstracts, 94Q.

Experimental[6]

Hydrolysis of Lactone-lactam of *endo-cis*-2,3-Dicarboxy-*endo*-5-amino-*endo*-6-hydroxynorbornane (I).—The lactone-lactam I (0.75 g., m.p. 191–192.5°) prepared as previously described[7] was refluxed in 20 ml. of 5% hydrochloric acid for 12 hr. The product was isolated as long white needles by concentrating the solution to a small volume and cooling. Recrystallization from water gave 0.61 g. (81%), m.p. 207–207.5°, alone and on admixture with an authentic sample of II.[7] Its infrared spectrum was identical with that of the authentic sample.

Anal. Calcd. for $C_9H_8O_4$: C, 60.00; H, 4.47. Found: C, 59.90; H, 4.75.

The aqueous solution remaining after the removal of II was made basic and steam distilled. The ammonia liberated was titrated with standard acid using a modified micro-Kjeldahl procedure[8] and 99% of the theoretical nitrogen content resulting from N-alkyl cleavage was detected.

Acid Hydrolysis of III.—The keto-lactam, III (0.10 g., m.p. 234–236°) prepared as previously described[2] was refluxed for 6 hr. in 20 ml. of 10% hydrochloric acid. After work-up as described above, a 70% yield of pure IV was obtained, m.p. 238–238.5° (reported[3] 239°), ν_{max}^{KBr} 1780, 1710, 1690 cm.$^{-1}$. Nitrogen analysis, as described above, indicated quantitative N-alkyl cleavage.

Conversion of IV into II.—The ketone, IV (0.048 g.), was added to a solution of 0.056 g. of sodium borohydride in 1 ml. of 50% ethanol. After standing at room temperature for 2 hr. the solution was made acidic with dilute hydrochloric acid and continuously extracted with ether for 10 hr. Evaporation of the ether left a solid which was heated in a sublimation tube at 170° and 40 mm. for 3 hr. The unszblimed residue (0.010 g.) was identical in melting point and infrared spectrum with an authentic sample of II.

Hydrolysis of N-*t*-butylisobutyramide.—N-*t*-Butylisobutyramide[9] (0.800 g., m.p. 115–117°) was added to a refluxing solution of 20 ml. of 20% hydrochloric acid in a closed system containing a gas burette. After 1 hr. 94 ml. (75% yield) of gas was evolved. The gas was identical in its infrared spectrum (10-cm. gas cell) with isobutylene.

(6) All melting points were taken on a Fisher–Johns melting point apparatus and are uncorrected. Analyses were done by Dr. A. Bernhardt (Mulheim, Germany). Infrared spectra were recorded on a Beckman IR-5 spectrophotometer.
(7) The authors thank Dr. P. Wilder, Jr., for an authentic sample of II.
(8) L. Miller and J. A. Houghton, *J. Biol. Chem.*, **159**, 373 (1945).
(9) Kindly supplied by Dr. R. C. Freeman, Monsanto Chemical Co., St. Louis, Mo.

The Effect of Coördination on the Reactivity of Aromatic Ligands. VII. Specific Ion Effects on Diazo Coupling Rates[1]

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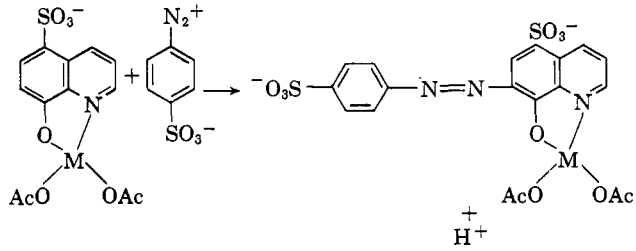
The effect of coördination to zinc ion on the rate of coupling of a phenolic chelating agent with a diazonium salt has been reported in a previous paper in this series.[2] The present work was undertaken to extend these rate studies to complexes of a number of other metal ions. The metal ions selected for this work were restricted to those which exhibit only one oxidation state in aqueous solution. In this way, side reactions of the metal ions with the diazonium salt were minimized.

(1) We wish to acknowledge, with thanks, the financial assistance of the U. S. Army Research Office (Durham).
(2) K. D. Maguire and M. M. Jones, *J. Am. Chem. Soc.*, in press.

The effect of coördination on the rate at which 8-hydroxyquinoline-5-sulfonic acid couples with diazotized sulfanilic acid has been determined for nine different metal ions. This coupling occurs at 7 position.



In most cases coördination of the 8-hydroxyquinoline-5-sulfonic acid reduces its rate of coupling, primarily through enormous decreases in the frequency factor. The activation energy is actually smaller for the chelates than for the free ligand. The free energy change for the formation of the activated complex has a minimum for the free ligand.

It is possible to show that the complexes themselves undergo the coupling reaction by determining the manner in which the rate of dye formation varies with the concentration of added metal ion. When a considerable increase in the concentration of metal ion has only a relatively small effect on the rate of dye formation, then it may safely be assumed that the rate measured is for the reaction of the complex and not for small traces of the free ligand. If only the phenolate ion (and not complexed phenolate) is assumed to react with the diazonium ion, then the factors which increase the concentrations of either the metal complex or the phenol, will decrease the rate of formation of the dye. In the present case, the concentrations of the 1:1 metal–phenolate complex will increase as more metal is added, so the rate of coupling should decrease indefinitely as metal is added *if the complexes do not couple*. That this is not the case can be clearly seen from the data in Table I.

The kinetic parameters derived from the rate constants are given in Table II. Several points are noteworthy, the most important being the great effect which coördination has on the entropy of activation. In all cases the entropy change on activation is less favorable for the complex than for the free phenolate ion. The changes are reasonably close to those expected if the charge on the reacting species (ligand) is decreased by unity.[3] Such a change is anticipated if the negative phenolate ion is tied up with the metal in a neutral chelate. The acetate ions of the buffer are probably coordinated to the central ion to furnish the rest of the charge needed for neutralization. It is quite striking that the activation energies for the reactions of the complexes are *all* lower than that of the free ligand.

A comparison of the experimental rates of coupling shows that the dye is actually produced most rapidly when cadmium(II) is present. This is because of the combination of low activation energy and suitable frequency factor for the complex of this particular ion. For all of the other complexes examined, the rate of production of the dye is less than that of the simple ligand in the absence of metal ions.

(3) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed., John Wiley and Sons Inc., New York, N. Y., 1961, pp. 142–150.

TABLE I

RATE CONSTANTS[a] FOR DIAZO COUPLING IN THE PRESENCE OF ADDED METALLIC SALTS

(The temperature is 15° unless otherwise specified)

	Metal–ligand ratio			
Metal ion	50:1	100:1	200:1	400:1
Lithium(I)	2.89	2.91	2.91	2.86
			0.55 (0°)	0.55 (0°
Sodium(I)	2.73	2.68	2.74	2.77
		0.55 (0°)		0.55 (0°)
Potassium(I)	2.82	2.82	2.82	2.80
			0.55 (0°)	0.55 (0°)
Magnesium(II)	2.82	2.83	2.75	2.66
	0.53 (0°)			0.53 (0°)
Cadmium(II)	7.16	6.89	7.05	6.93
	6.93	...	1.82 (0°)	7.37
				1.74 (0°)
Strontium(II)	2.83	2.77	2.82	2.97
		0.55 (0°)	0.55 (0°)	
Calcium(II)	2.36	2.52	2.60	2.51
			0.58 (0°)	0.60 (0°)
Barium(II)	...	2.90	2.81	...
			0.55 (0°)	0.58 (0°)
Aluminum(III)	0.0999	0.069	0.044	...
	0.167 (20°)			
	0.0565 (10°)			
None	...	3.93[b]		...

[a] All rate constants are pseudo first order, and are in terms of $\times 10^{-2}$ min.$^{-1}$. Each solution is buffered to pH 5. [b] Since only the phenolate anion undergoes coupling, the second-order rate constant must take this into consideration. When the corresponding rate constant based on the *phenolate* concentration is used it is found to be about 104 times greater than the constant for any of the chelates. See ref. 2 for further details.

TABLE II. DERIVED KINETIC PARAMETERS[a]

Metal	E_a, kcal./mole	A, sec.$^{-1}$	ΔS^*, e.u.	ΔF^*, kcal./mole
Lithium(I)	17.3	1.26×10^{12}	−3.0	18.1
Sodium(I)	16.7	4.21×10^{11}	−5.2	18.1
Potassium(I)	17.0	4.81×10^{11}	−5.0	18.4
Magnesium(II)	17.2	1.00×10^{12}	−3.5	18.2
Calcium(II)	15.0	2.15×10^{10}	−11.2	18.4
Strontium(II)	17.1	1.03×10^{12}	−3.4	18.1
Cadmium(II)	14.3	1.81×10^{10}	−11.5	17.5
Barium(II)	16.9	6.91×10^{11}	−8.8	19.3
Aluminum(III)	17.7	1.16×10^{11}	−7.8	19.9
Zinc(II)	13.4	7.7×10^{8}	−17.7	18.5[b]
None (phenolate)	19.3	9.7×10^{7}	23.8	12.5[b]

[a] All metals were chelated with oxine-5-sulfonic acid. [b] Taken from ref. 2.

For these reactions, the entropy of activation is apparently roughly proportional to the energy of activation. The entropies of activation are known to within 3 e.u. or better and the activation energies are reproducible to within 1 kcal. This kind of relationship is characteristic of a series of closely related reactions. It indicates that the nature of the activated complex does not differ profoundly for the free and the coördinated ligand. This supports the results of previous studies in this series. The reactivity sequence $—O^- > OM > OH$ indicates that the electronic density change which accompanies coördination of monovalent and divalent metal ions is much less than that which accompanies protonation.[2]

Experimental

Diazo Couplings.—The general procedure reported earlier[2] was used with only slight modification. A solution of the diazotized

sulfanilic acid of known concentration was prepared by diazotization and used as such. Previously the solid compound was isolated and weighed out for each run. The explosive character of the diazo salt made the use of a solution much more convenient. The reproducibility of the data was unaffected by the change and remained of the order ± 5%, with the greatest spread being 7%. In those cases where quenching of the reaction mixture in sulfuric acid resulted in a precipitate—(*e.g.*, calcium sulfate)—it was merely necessary to centrifuge the sample to obtain a clear supernatant liquid which could be used for the spectrophotometric estimation of the dye. The metals were added as their chloride or sulfate salts.

Rate Constants.—The rate constants are pseudo first-order rate constants obtained from the expression:

$$\text{Rate} = k_1 (\text{phenolate complex})(H^+)$$

The constant k_1 incorporates the product $k_2(RN_2^+)$, where (RN_2^+) is a constant excess of the diazonium salt and k_2 is the second order rate constant. This diazonium salt was present in fifty-fold excess. The concentration of the diazonium salt was 5.0×10^{-3} M while that of the phenol was 9.26×10^{-5} M in all of the runs. The second-order rate constants may be obtained from the relationship between the two rate constants and the concentration of diazonium salt.

Microbiological Transformations. III.[1] Reduction of the Steroid C-19 Aldehyde Group[2]

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Received November 15, 1962

Interconversions of ketone and hydroxyl groups were among the first described transformations of steroids by microörganisms, and many oxidations and reductions have been reported.[3] However, the conversion of 3-ketobisnor-4-cholen-22-al into 6β,11α,22-trihydroxybisnor-4-cholen-3-one has been the sole example of microbial reduction of a steroid aldehyde. We report herein the microbiological transformation of anhydrostrophanthidone (I) into 19-dihydroanhydrostrophanthidone (II). The conversion appears to constitute the first demonstration of reduction of a steroid *angular* aldehyde by a microörganism.

In preceding communications in this series,[1,4] we reported on the microbiological conversion of strophanthidin into anhydrostrophanthidone (I). For continuing studies, I was the substrate of choice, in view of the ease of detection of Δ^4-3-oxosteroid derivatives on paper chromatograms.

When I was incubated with *Penicillium thomii*, a more polar compound, m.p. 247–251°, was obtained in 55% yield. Analysis afforded figures in good agreement with the formula $C_{23}H_{30}O_5$. The ultraviolet spectrum showed λ_{max}^{MeOH} 219 mμ (Δ α,β-lactone), and the infrared spectrum showed bands at 2.92 μ (OH), 5.60 μ (Δ α,β-lactone), 6.06 and 6.20 μ (Δ α,β-ketone). Acetylation with acetic anhydride–pyridine afforded a monoacetate derivative. On the basis of the foregoing facts, the transformation product was characterized as II and the monoacetate as IV. The structural assignment was confirmed by synthesis of II by catalytic oxidation of strophanthidol (III) in the presence of platinum and oxygen. Direct comparison of the catalytic oxidation product and its acetate with the microbiological transformation product and its acetate indicated the identity of the respective compounds.

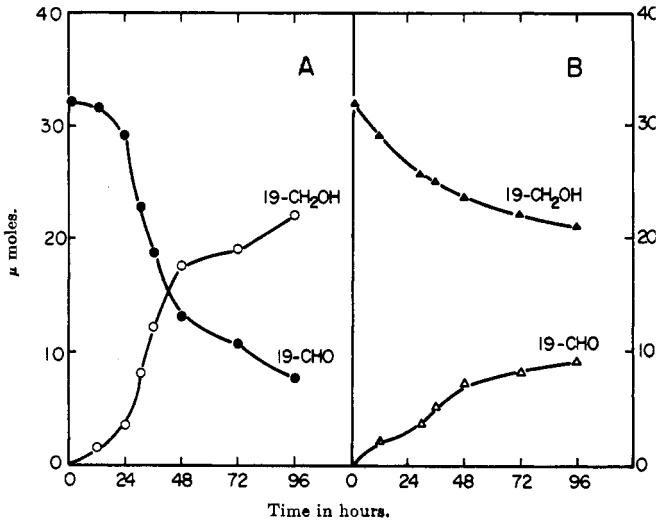
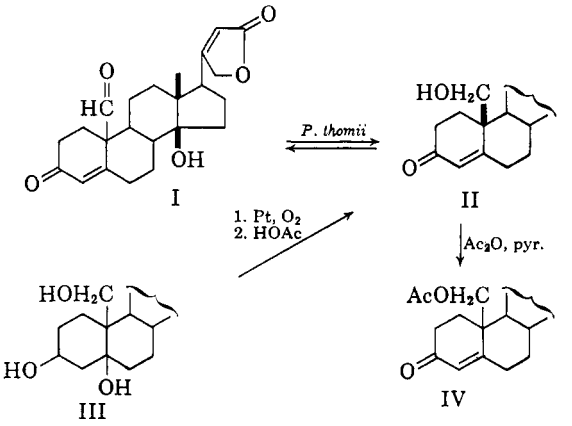


Fig. 1.—Interconversion of anhydrostrophanthidone (I) and 19-dihydroanhydrostrophanthidone (II) by *Penicillium thomii*. (A)—I as the substrate. (B)—II as the substrate.



The transformation is of biochemical interest because the reversal of the reaction parallels a possible biosynthetic step in the formation of estrogens from androgens. Recent studies with human placental microsomes support the sequence Δ^4-androstenedione → 19-hydroxyandrostenedione → 19-oxoandrostenedione ⇢ estrone for the steps in biological estrogen formation.[5] To demonstrate the reversibility of the microbial transformation, I and II were separately exposed to *P. thomii* under identical conditions. Fig. 1A and 1B show that approximately the same equilibrium

(1) Part II in the series: S. M. Kupchan, C. J. Sih, N. Katsui, and O. El Tayeb, *J. Am. Chem. Soc.*, **84**, 1752 (1962).

(2) This investigation was supported in part by research grants (H-2275, A-4069) from the National Institutes of Health.

(3) *Cf., e.g.*, P. Talalay, *Physiol. Rev.*, **37**, 362 (1957).

(4) C. J. Sih, S. M. Kupchan, O. El Tayeb, and A. Afonso, *J. Med. Pharm. Chem.*, **5**, 629 (1962).

(5) T. Morato, M. Hayano, R. I. Dorfman, and L. R. Axelrod, *Biochem. Biophys. Res. Comm.*, **6**, 334 (1961).

(6) P. Talalay and P. I. Marcus, *J. Biol. Chem.*, **218**, 675 (1956).