Stannic Chloride Adducts with Lactones

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RECEIVED JUNE 17, 1954

The formation of hydrobromides and hydrochlorides of coumarin, a lactone, has been reported,[2] as well as stannic chloride adducts of esters,[3] and aliphatic,[3] aromatic[4] and cyclic ethers.[5] Recently qualitative tests have demonstrated that dilute solutions of lactones in pentane form precipitates when solutions of stannic chloride in pentane are added. Three authentic lactones have been subjected to this test and the precipitates have been analyzed quantitatively for the content of tin by the method described earlier.[5] In each case the analysis corresponds to the formation of an adduct between two molecules of the lactone and one of stannic chloride as shown in Table I.

TABLE I

ANALYSIS OF ADDUCTS FOR STANNIC CHLORIDE

Lactone	1 Mole of lactone per mole of $SnCl_4$	2 Moles of lactone per mole of $SnCl_4$	Found
Coumarin	64.06	47.14	47.75
			47.45
			47.45
			47.10
Lactone of 2-hydroxybiphenyl-2′-carboxylic acid (6-dibenzopyrone)	57.04	39.90	40.76
			40.67
Phthalide	66.01	49.27	48.44
			47.89

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(2) W. H. Perkin, *Ann.*, **157**, 116 (1871).

(3) P. Pfeiffer and O. Halperin, *Z. anorg. Chem.*, **87**, 335 (1914).

(4) H. H. Sisler and co-workers, THIS JOURNAL, **70**, 3818 (1948).

(5) *Ibid.*, p. 3821.

(6) J. Entel, C. H. Ruof and H. C. Howard, *ibid.*, **74**, 441 (1952).

COMMUNICATIONS TO THE EDITOR

THE RADIATION-INDUCED OXIDATION OF FERROUS ION[1]

Sir:

In the presence of dissolved molecular oxygen, ferrous ion in 0.8 N H_2SO_4 is more rapidly oxidized by ionizing radiations than in the absence of oxygen. The ratio of these rates provides important information regarding the role of molecular oxygen, and the variation of the ratio with the linear ion density characteristic of the radiations is a measure of the molecular yield.

Hart[2] has reported a value of 2.86 for the ratio of the rate of radiation-induced oxidation of ferrous ion in the presence and absence of oxygen, and has compiled reported values ranging from 2.5 to 4.0 for γ-rays and hard X-rays. Rigg, Stein and Weiss[3] have reported a minimum value of 2.0. Recently we have determined the value of this ratio for Co^{60} γ-rays to be 1.88 ± 0.04, as shown in Table I. This value is in excellent agreement with a value of 1.9 calculated on the basis of the mechanism proposed by Weiss[3] when it is modified to include the molecular hydrogen yield reported by Allen.[4]

Experimental procedures and a discussion of the sources of discrepancy amongst the various experimental values will be published.

TABLE I

	Observed: 3×10^{18} e.v./ml./hr. Ratio[a]	Observed: 1.2×10^{19} e.v./ml./hr.	Calcd.
$(dFe^{+++}/dt)_{O_2}/(dFe^{+++}/dt)_{H_2O}$	1.88 ± 0.04		1.90
$(dFe^{+++}/dt)_{O_2}/(dO_2/dt)_{Fe}$	4.15 ± 0.1	4.14 ± 0.2	4.25

[a] Fe^{+++} determined spectrophotometrically at 305 mμ; O_2 determined polarographically.

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RECEIVED SEPTEMBER 30, 1954

PURIFICATION AND STRUCTURE OF β-CORTICOTROPIN

Sir:

On behalf of my many colleagues in the Research Division[1] I wish to report that one of the physiologically active components of corticotropin from hog anterior pituitary has been separated in pure form and a tentative structure has been deduced. Seven other distinct proteins of equally high corticotropin activity were also isolated in lesser yields.

"Clinical" ACTH[2] prepared by the acetic acid

(1) Supported in part by U. S. Atomic Energy Commission Contract #AT(30-1)-1186 and in part by the The Nutrition Foundation, Inc., New York, N. Y.

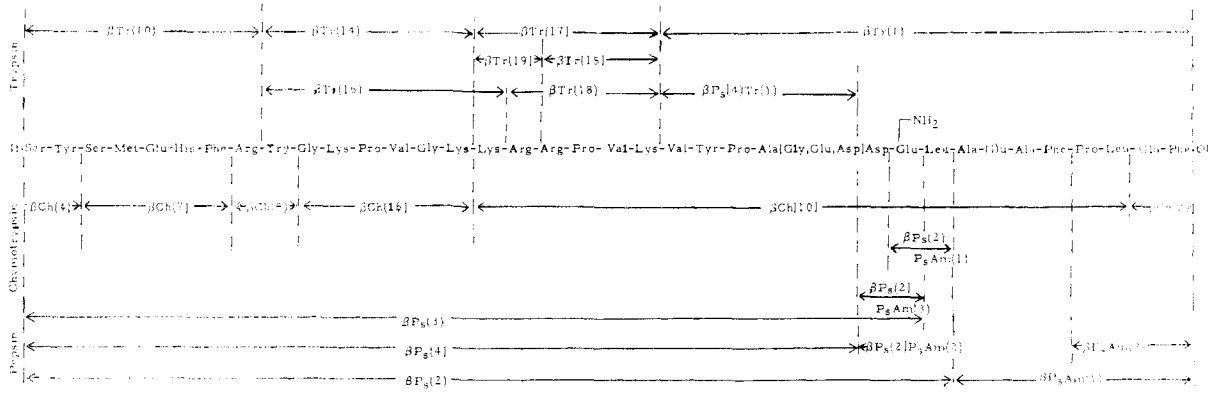
(2) E. J. Hart, THIS JOURNAL, **73**, 1892 (1951).

(3) T. Rigg, G. Stein and J. Weiss, *Proc. Roy. Soc. (London)*, **211A**, 375 (1952).

(4) H. A. Schwarz, J. T. Lossee and A. O. Allen, THIS JOURNAL, **76**, in press (1954).

(1) Stamford Laboratories: R. G. Shepherd, K. S. Howard, A. R. Cacciola, S. B. Davis, D. S. Davies, E. A. Eigner, J. P. English, B. M. Finn, J. H. Meisenhelder, N. E. Shakespeare, S. D. Willson. Lederle Laboratories: A. W. Moyer, R. A. Brown, R. G. Child, M. C. Davies, C. C. Scrobola, J. van der Scheer.

(2) Supplied by Dr. David Klein, Wilson and Co., Inc., and by Dr. H. R. Cox, Lederle Laboratories, Research Division, American Cyanamid Company.



method of Payne, *et al.*,[3] was further purified by the oxycellulose procedure of Astwood, *et al.*[4] The oxycellulose ACTH (OC-ACTH) was resolved into eight components by an extended countercurrent distribution (CCD) using the system: 6% acetic acid containing 3.5% NaCl *vs.* *n*-BuOH. The partially resolved α and γ fractions as well as δ (K = 0.03)[5,6] were removed from the 200 tube CCD machine at n = 200, and β (K = 0.95), which was the major component (*ca.* 33%), was recycled for 720 additional transfers. At n = 920 the β was judged pure by its theoretical curve shape and by measured distribution coefficients (K) across the peak. The α and γ fractions were each recovered[7] and redistributed in the same system. After 400–500 transfers the following active components were separated: α_1, K = 12–14; α_2, K = 4–5; α_3, K = 1.5–2.0; γ_1, K = 0.35; γ_2, K = 0.16 and γ_3, K = 0.27.[5,6]

β-ACTH was selected for chemical study, since it is present in largest quantity in the material used clinically.

Amino acid analysis of acid hydrolysates by Dowex-50 columns[8] along with a direct tryptophane determination[9] gave the following amino acid empirical formula for β-ACTH

$$Ala_3Arg_3Asp_2Glu_5Gly_3His_1Leu_2Lys_4Met_1Phe_3Pro_4Ser_2Try_1\text{-}Tyr_2Val_3(NH_3)_1$$[10]

Ultracentrifuge data[11] calculated by the Archibald method[12] gave a free base molecular weight of 4500, which is in excellent agreement with the 4566 required by the above formula.

In order to elucidate the structure of β-ACTH with the decigram amounts available, specific methods of cleavage were selected rather than the random hydrolysis method. The steps involved in this study were: (a) specific cleavage by enzyme digestions[13]; (b) resolution of peptides from these digestions by CCD; (c) characterization of these peptide fragments by amino acid analysis, Edman stepwise degradations,[14] and time studies with carboxypeptidase.

Pepsin digestion split β-ACTH into large basic peptides plus a group of small acidic peptides which were adsorbed on Amberlite IRA-400 Acetate. $\beta P_sAm(1)$ and $\beta P_sAm(2)$ were resolved from this adsorbed group by two-dimensional paper chromatography. The mixture of unadsorbed basic peptides was separated by a 450-transfer CCD, using 0.5% trichloroacetic acid (TCA) *vs.* *n*-BuOH,[15] into active peptides: $\beta P_s(2)$, K = 1.56; $\beta P_s(3)$, K = 0.23; and $\beta P_s(4)$, K = 0.32.[5,6] $\beta P_s(2)$ digested with a higher concentration of pepsin gave only $\beta P_s(4)$.

The trypsin and chymotrypsin digests of β-ACTH (200 mg.) were resolved by 8,000 and 10,000 transfer distributions using the system: 20% acetic acid *vs.* *n*-BuOH. The major trypsin cleavages of β led to βTr(1), βTr(10), βTr(14) and βTr(17), which accounted for greater than 90% of the starting material. The chymotrypsin products shown also accounted for most of the starting β. All of the peptides gave amino acid mole ratios which uniquely located them in the indicated structure. The structures of the following peptides were completely elucidated: $\beta P_sAm(1)$, $\beta P_sAm(2)$, $\beta P_s(2)$-

(3) R. W. Payne, M. S. Raben and E. B. Astwood, *J. Biol. Chem.*, **187**, 719 (1950).

(4) E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951).

(5) Corticotropin activities, by the ascorbic acid depletion method [M. A. Sayers, G. Sayers and L. A. Woodbury, *Endocrinology*, **42**, 379 (1948)], for OC-ACTH, α_1, α_2, α_3, β, (γ_1 + γ_3), (γ_2 + γ_3), δ, $\beta P_s(2)$, $\beta P_s(3)$ and $\beta P_s(4)$ were consistently 80–100 units per mg. with occasional assays going as high as 450 units per mg.

(6) Intermedin assays (normal *Rana pipiens* frogs) on α_1, α_2, α_3, β, (γ_1 + γ_3), (γ_2 + γ_3), $\beta P_s(2)$, $\beta P_s(3)$ and $\beta P_s(4)$ gave a maximum melanophore expansion of 4–6 hours duration at *ca.* 5γ per 100 g. dosages. The persistence of a nearly constant intermedin to corticotropin ratio in these samples makes it highly unlikely that the melanophore action is due to an impurity. The δ fraction gave the same intermedin response at a 0.15γ per 100 g. level. After corticotropin inactivation by 0.1 N NaOH at 100°, for 20 minutes, the intermedin activities of the first group were increased to the level given by alkali-treated δ (maximum response at *ca.* 0.1 γ per 100 g.). The melanophore expansion of all alkali-treated fractions lasted several days.

(7) Recovered salt-free by a short CCD with added trichloroacetic acid.

(8) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 163 (1951).

(9) J. R. Spies and D. C. Chambers, *Anal. Chem.*, **20**, 39 (1948).

(10) Microbiological amino acid assays on these acid hydrolysates were in good agreement with these data. These tests were specific for the L configuration in all cases except alanine and aspartic acid (courtesy of A. C. Dornbush, Lederle Laboratories, Research Division, American Cyanamid Company).

(11) Personal communication from Dr. R. A. Brown, Lederle Laboratories, Research Division, American Cyanamid Company.

(12) W. J. Archibald, *J. Phys. Colloid Chem.*, **51**, 1204 (1947).

(13) This method cannot be considered of general use unless digestions by two or more enzymes are thoroughly investigated in order that possible transpeptidation, as suggested by the work of S. G. Waley and J. Watson, *Nature*, **167**, 361 (1951), *Biochem. J.*, **57**, 529 (1954), and K. Blau and S. G. Waley, *ibid.*, **57**, 538 (1954), can be recognized.

(14) A modification of the method of P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).

(15) The 0.5% TCA *vs.* *sec*-BuOH system [F. A. Kuehl, Jr., M. A. P. Mesinger, N. G. Brink and K. Folkers, THIS JOURNAL, **75**, 1955 (1953)] did not separate this mixture.

P_sAm(1), βCh(2), βCh(4), βCh(5), βCh(7), βTr-(10), βTr(14), and βTr(17). Sufficient data on the remaining peptides were obtained to show their compatibility with the assigned structures.[16]

The data on peptides βTr(1) and βP_s(4)Tr(1) suggest the structure indicated in the parentheses of the structure. Research designed to remove this uncertainty is underway and will be reported along with the experimental details of this work in publications now in preparation.

(16) The N-terminal sequence H·Ser-Tyr-Ser-Met-Glu-His-Phe- has been shown to occur in *sheep* corticotropin [J. Harris and C. H. Li, THIS JOURNAL, **76**, 3607 (1954)]. The H·Ser-Tyr- N-terminal and Pro-Leu-Glu-Phe·OH C-terminal orders for corticotropin A from hog pituitaries were reported by W. A. Landmann, *et al.*, *ibid.*, **75**, 4370 (1953), and W. F. White, *ibid.*, **75**, 4877 (1953).

CHEMOTHERAPY DEPARTMENT
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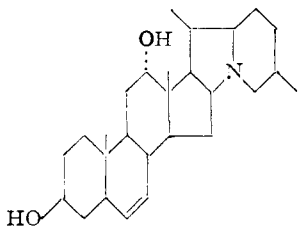
RECEIVED SEPTEMBER 20, 1954

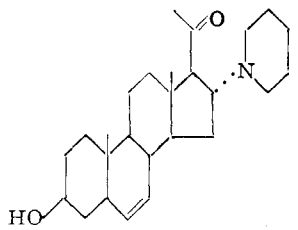
SYNTHETIC STEROIDAL CARDIOACTIVE AMINES

Sir:

In synthetic approaches to the structure of steroidal alkaloids such as rubijervine,[1] I, we have prepared various 16-aminopregnenolones, among them 16α-piperidino-5-pregnen-3β-ol-20-one, II.



I



II

5,16-Pregnadien-3β-ol-20-one was treated with excess piperidine and aqueous potassium hydroxide to give II, which was isolated in two crystalline forms, m.p. 149–151° and 160–162°; $[\alpha]^{25}D$ −23.5°, −24.7° (dioxane). *Anal.* Calcd. for $C_{26}H_{41}O_2N$: C, 78.14; H, 10.34; N, 3.51. Found: C, 78.28, 78.17; H, 10.22, 10.14; N, 3.60, 3.51, respectively. Both forms showed the same infrared spectrum and gave the same hydrochloride, m.p. 240–242° (dec.), $[\alpha]^{25}D$ +8.7° (95% EtOH). *Anal.* Calcd. for $C_{26}H_{41}O_2N$·HCl: N, 3.21; Cl, 8.13. Found: N, 3.21; Cl, 8.06.

Although II only superficially resembles I, it shows some hypotensive action in dogs at a dose of 1–2 mg./kg.,[2] which is the order of activity of I.[3] In contrast to earlier experience,[4] however, II, although it contains a tertiary nitrogen similar to the known hypotensive veratrum ester alkaloids, also exhibits the bradycrotic and specific contra-accelerator action previously found only with the secondary steroidal alkamines such as jervine.[2,4]

Similarly, addition of cyclohexylamine to 5,16-pregnadienolone gave 16α-cyclohexylamino-5-pregnen-3β-ol-20-one, III, m.p. 151–152°, $[\alpha]^{25}D$ −29.8° (dioxane). *Anal.* Calcd. for $C_{27}H_{43}O_2N$: C, 78.40; H, 10.48; N, 3.39. Found: C, 78.35; H, 10.46; N, 3.53. Catalytic hydrogenation of III using platinum in acetic acid gave 16α-cyclohexylamino-allopregnane-3β,20ζ-diol (IV), m.p. 179–180.5°, $[\alpha]^{25}D$ −58.1° (dioxane). *Anal.* Calcd. for $C_{27}H_{47}O_2N$: C, 77.64; H, 11.34; N, 3.35. Found: C, 77.57; H, 11.30; N, 3.05.

These secondary amines, III and IV, show the contraaccelerator effect typical of secondary alkamines, but in addition have activity against arrhythmias of the heart. Thus IV shows a potency about five times that of jervine[5] against the chromotropic effect of epinephrine in the isolated heart, and also in intact animals; and a potency about four times that of quinidine against methacholine induced auricular arrhythmias in dogs.[2]

Further investigations of pharmacologically active synthetic steroidal amines are continuing and will be reported in detail at a later date.

(1) Y. Sato and W. A. Jacobs, *J. Biol. Chem.*, **179**, 623 (1949).

(2) We are indebted to Dr. O. Krayer, Department of Pharmacology, Harvard Medical School, for his continued interest and advice, and to Drs. S. Margolin and G. Lu, Pharmacology Department, Schering Corporation, for the pharmacological results which will be published elsewhere.

(3) G. L. Maison, E. Gotz and J. W. Stutzman, *J. Pharmacol. and Exper. Therap.*, **103**, 74 (1951).

(4) O. Krayer and L. H. Briggs, *Brit. J. of Pharmac. and Chemo.*, **5**, 118, 517 (1950); F. C. Uhle, THIS JOURNAL, **73**, 883 (1951).

(5) O. Krayer, F. C. Uhle and P. Ourisson, *J. Pharmacol. and Exper. Therap.*, **102**, 261 (1951).

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RECEIVED OCTOBER 7, 1954

A QUANTITATIVE APPROACH TO ION EXCHANGE CATALYSIS

Sir:

The quantitative interpretation of ion exchange catalysis is simplified by treating the pore liquid of the resin, in which the reaction occurs, as a homogeneous system, and comparing it with a homogeneous solution containing dissolved electrolyte as catalyst, both at equal concentration of the catalyst ion, the supernatant solution in case of ion exchange catalysis being used merely as a means for determining the necessary quantities. Comparison of the rate constants and activation energies in both systems will then reveal special influences of the resin other than adsorption phenomena which can be accounted for separately.

The rate determining step in ion exchange catalysis can be the velocity of the reaction, or the diffusion within the resin. We deal first with reaction controlled catalysis.

For the simple case of a first order reaction without reverse reaction

$$AB \longrightarrow A + B \qquad (1)$$

(for instance sucrose inversion) the reaction rate in a homogeneous solution is given by

$$-dc/dt = kc \qquad (k = f(c_{cat.}) \approx k'c_{cat.}) \qquad (2)$$

where c is the concentration of the reactant AB. The rate constant k is a function of the catalyst concentration c_{cat} and approximately proportional to c_{cat}. An analogous equation may be written for the pore liquid, denoting all quantities referring to pore liquid with bars. The assumption of reaction controlled catalysis implies that $\bar{c}$ has its