saponification, gave on acidification the lactone-dicarboxylic acid IIIa ($R^1 = R^2 = CO_2H$, $R^3 = H$)[3] which was transformed, through reaction of the diacid chloride acetate IIIa ($R^1 = R^2 = COCl$, $R^3 = Ac$) with dimethylketene acetal,[5] into the diketo-lactone IIIa ($R^1 = R^2 = COCH_3$, $R^3 = Ac$) [isomer a, m.p. 172–173°; isomer b, m.p. 145–146°].[3] Rearrangement with trifluoroperacetic acid[6] afforded the triacetate IIIa ($R^1 = R^2 = OAc$, $R^3 = Ac$) [isomer a, m.p. 140–141°; isomer b, m.p. 142–143°][3] which, on mild saponification followed by N-bromoacetamide oxidation and reacetylation, gave the 3-ketodiacetate [isomer a, m.p. 135–136°; isomer b, m.p. 147–148.5°].[3] Bromination and dehydrobromination[7] afforded the unsaturated ketone IIIb ($R^1 = R^2 = OAc$) [isomer a, m.p. 183–184°; isomer b, m.p. 154–155°],[3] which was converted[8] into the ketal IIIc ($R^1 = R^2 = OAc$) [isomer a, m.p. 155–156°; isomer b, m.p. 175–176°].[3] Saponification gave the diol IIIc ($R^1 = R^2 = OH$), [isomer a, m.p. 185–186°; isomer b, m.p. 205–207°] which was transformed, by selective reaction with 2,5-dimethylbenzenesulfonyl chloride in pyridine, into the monoester IIIc ($R^1 = OH$, $R^2 = OSO_2C_6H_3Me_2$). Oxidation with Sarett's reagent converted the C_{20} hydroxyl to ketone; thus both epimers yielded the same product[3] which, with potassium *t*-butoxide, underwent cyclization[9] to the keto-lactone IVc, m.p. 194–196°. This substance was partially isomerized by base into the 17β epimer Vc, m.p. 210–214° (reported,[1a] 202–208°). The latter substance and the corresponding ketone Vb, m.p. 215–219° (reported,[1a] 218–220°) were shown, by infrared and mixed m.p. comparisons, to be identical with authentic specimens.[10] Vc has been converted into aldosterone.[1a]

Although our main objective was thus realized we hoped to obviate certain difficulties attending the last stages of the synthesis due to the β-oriented C_{17} side-chain[1a] by operating in the 17α series. With two mole-equivalents of lithium aluminum hydride IVc was selectively reduced to the lactol VIc ($R^1 = H$, $R^2 = CHOHCH_3$), m.p. 190–194°, which was hydrolyzed to VIb ($R^1 = H$, $R^2 = CHOHCH_3$), m.p. 127–133°, then treated with methanol and acid to yield the lactol ether VIb ($R^1 = CH_3$, $R^2 = CHOHCH_3$), m.p. 168–169.5°. Oxidation with Sarett's reagent afforded the ketone VIb ($R^1 = CH_3$, $R^2 = COCH_3$), m.p. 166–168°, which was converted[11] into the 21-acetoxy compound VIb ($R^1 = CH_3$, $R^2 = COCH_2OAc$), m.p. 138–140°. Hydrolysis of the lactol ether with 70% acetic acid gave *dl*-17α-aldosterone-21-acetate, VIb ($R^1 = H$, $R^2 = COCH_2OAc$), m.p. 166–170°. The infrared spectrum was identical with that[10] of material obtained by partial isomerization of the 17β-epimer.[1a] Treatment of VIb ($R^1 = H$, $R^2 = COCH_2OAc$) with potassium carbonate in aqueous methanol gave the C_{17} epimeric mixture [1a] from which *dl*-aldosterone readily was isolated. The product was identified with authentic material[10] by paper chromatographic behavior, infrared spectroscopy and physiological activity.[12,13]

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(13) We are grateful to the Sterling-Winthrop Research Institute, the Upjohn Company, the Wisconsin Alumni Research Foundation and the National Science Foundation for assistance.

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RECEIVED APRIL 18, 1958

THE COMPLEX FORMED FROM COBALT HYDROCARBONYL AND BUTADIENE

Sir:

Although Prichard has reported the preparation of a complex between butadiene and dicobalt octacarbonyl under Oxo conditions,[1] only an analysis for cobalt and an analysis by the Orsat technique of the sulfuric acid decomposition products were given.

We have now been able to prepare and study the stereochemical configuration of the complex formed between butadiene and cobalt hydrocarbonyl in the absence of Oxo conditions.

Potassium cobaltcarbonylate was made to react with glacial acetic acid and liquid butadiene in a high pressure vessel. After twelve hours, a red-brown liquid, distilling at 33–35° at less than 1 mm. pressure, was obtained.

Analysis showed it to have the empirical composition $Co(CO)_3C_4H_7$. In benzene it gave a molecular weight of 195, compared to a calculated molecular weight of 198. The compound is diamagnetic and contains no acidic hydrogen.

The infrared and ultraviolet absorption characteristics indicate the disappearance of the conjugated diolefin structure and the appearance of a structure producing absorption characteristics similar to those of *cis* monoölefins. The sharp peak at 703 cm.$^{-1}$ which in the cobalt hydrocarbonyl has been assigned[2,3] to the hydrogen vibrations also has disappeared.

However, carbon monoxide could react with butadiene to give cyclopentanone, which might have some aromatic character and give a complex similar to cyclopentadiene bis-carbonyl cobalt; however, the infrared data do not seem to be in line with such a structure.

Experimental details and a reinterpretation of the mechanism of the oxo reaction[4,5,6] will be reported shortly.

Thanks are due to Drs. L. Orgel and H. W. Sternberg for discussions.

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berg for stimulating discussions, and to Esso Standard Oil Company for financial support.

(7) Esso Standard Oil Company Fellow, 1955–57.

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RECEIVED MARCH 25, 1958

ISOLATION OF MELATONIN, THE PINEAL GLAND FACTOR THAT LIGHTENS MELANOCYTES[1]

Sir:

During the past forty years investigators have reported that injection of pineal gland extracts into tadpoles, frogs, toads and fish produces lightening of skin color.[2–4] Recently it was found that such extracts, by causing aggregation of melanin granules within the melanocytes of isolated pieces of frog skin, reverse the darkening effect of the melanocyte stimulating hormone (MSH).[5] We wish to report isolation from beef pineal glands of the active factor that can lighten skin color and inhibit MSH. It is suggested that this substance be called *melatonin*.

Fifty grams of powdered lyophilized beef pineal glands[6] was extracted with petroleum ether for two hours in a soxhlet extractor. The defatted powder was mixed with 900 ml. water in a Waring Blendor. After centrifugation at 16,000 × *g* for 30 minutes the supernatant was extracted with 900 ml. ethyl acetate. The ethyl acetate layer was concentrated *in vacuo* at 50° and subjected to distribution in a 30-tube countercurrent apparatus with the solvent system ethyl acetate, heptane, water (1:1:2 v./v.). Tubes 8–15 were combined. The water layer was extracted twice with 80 ml. portions of ethyl acetate. All the organic solvent extracts were combined and evaporated to dryness *in vacuo* at 50°. The residue was sublimed at 80° *in vacuo*. The sublimate was transferred with ethanol to Whatman No. 1 filter paper and chromatographed by descending technique with solvent system benzene, ethyl acetate, water (19:1:20). A test strip on reaction with Ehrlich reagent (*p*-dimethylaminobenzaldehyde) showed a blue spot at R_f 0.38. The unreacted strip was cut into sections and eluted with ethanol. Bioassay was performed using isolated *Rana pipiens* skin darkened with caffeine. The lightening effect of the test substance on the melanocytes was measured photometrically with transmitted light. This revealed that 95% of recoverable biologic activity was present at the position of the blue spot. Spectrophotofluorometric analysis of the active eluate showed a single fluorescent peak at 3380 Å. which was excited maximally at 2950 Å. Ultraviolet absorption analysis showed a maximum at 2725 Å. with inflections at 2950 and 3080 Å. The fluorescence and ultraviolet absorption were characteristic of hydroxyindoles.

The active material was rechromatographed and eluted in three successive solvent systems. The biologic activity, characteristic fluorescence, and blue color with Ehrlich reagent remained exclusively together as a spot on these chromatograms. The solvent systems were isopropyl alcohol, concentrated ammonium hydroxide, water (16:1:3) R_f = 0.83; 1-butanol, acetic acid, water (4:1:5) R_f = 0.87; isopropyl alcohol, concentrated ammonium hydroxide, water (10:1:1) R_f = 0.86.

In preventing darkening of frog skin by MSH, melatonin, the active pineal gland factor, was at least 100 times as active on a weight basis as adrenaline or noradrenaline, 200 times as active as triiodothyronine and 5,000 times as active as serotonin.[5] Melatonin had no adrenaline nor noradrenaline-like activity on rat uterus and no serotonin-like activity on clam heart. No melatonin activity was detected in beef pituitary, hypothalamus, thymus, thyroid, adrenal, ovary, testis or eye.

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RECEIVED MARCH 28, 1958

THE STRUCTURE OF BOVINE CORTICOTROPIN[1,2]

Sir:

The isolation of bovine corticotropin, a 39 amino-acid polypeptide possessing ACTH activity, has been reported from this laboratory[3]; its amino acid composition is identical with that found for ovine α-corticotropin but different from that of the porcine hormone. It was further demonstrated that the porcine, ovine and bovine hormones possess identical N- and C-terminal residues. We wish to report herein the complete amino acid sequence of bovine corticotropin. It will be noted that there is a difference in certain portions of the amino acid sequence among the hormones of all three species.

By means of the paper-strip modification[4] of the phenyl isothiocyanate method,[5] the N-terminal amino acid sequence Ser.Tyr.Ser.Met.Glu. . . . was established for bovine corticotropin. The rate of release of amino acids from the carboxyl end of the peptide hormone by the carboxypeptidase procedure[6] indicated the sequence . . .Leu.Glu.Phe at the C-terminus.

Chymotryptic digests of the hormone (substrate/enzyme = 100/0.6 (w./w.), *p*H 9.0, 40°, for 24 hours) were fractionated by zone electrophoresis on paper for 7 hours at 200 volts with a collidine–acetic acid buffer of *p*H 7; after elution of each band, the peptide fragments were further purified by paper chromatography in either *n*-BuOH/

(1) This investigation was supported by grants from the American Cancer Society and the United States Public Health Service.

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(6) We are grateful to the Armour Laboratories for supplying us with several kilograms of beef pineal glands.

(1) Paper XIV of the corticotropins (ACTH) series; for Paper XIII, see C. H. Li, R. D. Cole, D. Chung and J. Leonis, *J. Biol. Chem.*, **227**, 207 (1957).

(2) This work is supported in part by the U. S. Public Health Service (G-2907) and the Albert and Mary Lasker Foundation.

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