Wir nehmen an, dass das durch Cobragift gebildete Anaphylatoxin das gleiche ist wie das durch Dextran u.a. Aktivatoren freigelegte. Diese Annahme gründet sich auf folgende Befunde: Beide Anaphylatoxine zeigen am isolierten Meerschweichendarm den gleichen Wirkungsablauf, Tachyphylaxie mit Erholung[7] und gekreuzte Tachyphylaxie. Rinder-, Pferde- und Menschenplasma, die mit Dextran u.a. kein Anaphylatoxin bilden, tun dies auch nicht mit Cobragift. Eine Ausnahme bildet Schweineplasma. Es gewinnt nach Inkubation mit Cobravollgift oder dem gereinigten Cobraferment eine starke Anaphylatoxinaktivität, die die gleichen Eigenschaften wie Ratten-Anaphylatoxin zeigt. Offenbar enthält Schweineplasma Substrat für Anaphylatoxin, aber kein vollständiges anaphylatoxinbildendes System.

Akzeptiert man die noch nicht bewiesene, aber wahrscheinliche Vermutung, dass die verschiedenen Methoden der Anaphylatoxinbildung zum gleichen Produkt führen, so würde dies bedeuten, dass auch die klassische Herstellung von Anaphylatoxin durch Kontaktsubstanzen ein fermentativer Spaltprozess ist, der durch die angewandten Substanzen aktiviert wird[8].

[7] K. D. FRIEDBERG, G. ENGELHARDT und F. MEINEKE, Int. Arch. Allergy *22*, 166 (1963).

Summary. Cobra venom contains an anaphylatoxin-forming principle. This component has been purified by gel filtration and ion exchange chromatography. It has been obtained free from proteolytic or hemolytic activity as well as from phospholipase A. It seems to be an enzyme that splits the anaphylatoxin from its inactive precursor.

W. VOGT und G. SCHMIDT

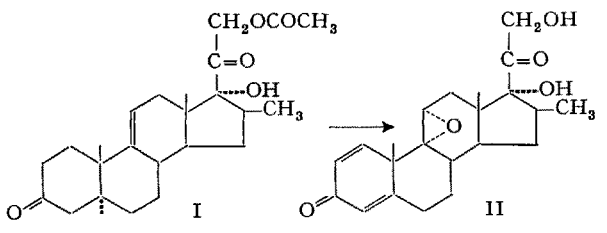
Pharmakologische Abteilung der Medizinischen Forschungsanstalt der Max-Planck-Gesellschaft, Göttingen (Deutschland), 16. Dezember 1963.

[8] *Nachtrag* (bei der Korrektur). Die Annahme, dass die Behandlung von Rattenplasma mit dem Cobraferment zum gleichen Anaphylatoxin führt wie nach Sephadex-Aktivierung, wurde inzwischen durch folgende Befunde weiter bestätigt: 1. Im Plasma von Ratten, die 140 min und 15 min vor der Blutentnahme das Cobraferment i.v. erhielten, konnte mit Sephadex kein Anaphylatoxin gebildet werden; 2. Wird Rattenplasma erst mit Sephadex und dann mit Cobraferment inkubiert, so entsteht nicht mehr Anaphylatoxin-Aktivität als nach Behandlung mit Cobraferment allein. Danach wird bei beiden anaphylatoxinbildenden Prozessen das gleiche Substrat verbraucht.

An Epoxidation by *Corynebacterium simplex*

An earlier communication from our laboratories[1] reported the microbiological dehydrogenation of 16-β-methyl-dihydrocortisone in the positions 1 and 4 by means of fermentation with *Corynebacterium simplex*. In continuation of this work further studies were undertaken concerning the dehydrogenation capacities of this bacterium strain in relation to other 16β-methyl steroid compounds.

In this paper we wish to report the conversion of 16β-methyl-5α-Δ9(11)-pregnene-17α,21-diol-3,20-dione-21-acetate (I) into 16β-methyl-Δ1,4-pregnadiene-9α,11α-epoxy-17α,21-diol-3,20-dione (II) by a strain of *Corynebacterium simplex* (ATCC 6946).



Microbiological epoxidations of double bonds have been obtained previously[2]. This reaction occurred only when unsaturated steroids were incubated with microorganisms capable of hydroxylating the corresponding saturated position. For instance *Curvularia lunata* or *Cunninghamella blakesleana* will convert Δ4,9(11)-pregnadiene-17α,21-diol-3,20-dione into the corresponding 9β,11β epoxide. The same two moulds, together with *Helicostylum piriforme*, *Mucor griseocyanus* or *Mucor parasiticus* were able to transform Δ4,14-pregnadiene-17α,21-diol-3,20-dione into the corresponding 14α,15α epoxide.

Up to date *Corynebacterium simplex* has been known only for its dehydrogenation capacity. The epoxidation of compound I in the position 9α,11α represents a new type of microbial conversion by this microorganism. This bacterium could therefore be considered as a potential 11α-hydroxylator. It is noteworthy that the epoxidation is accompanied by a dienization in the positions 1 and 4 of ring A. Besides compound II some fermentations yielded an additional steroid compound which appeared most probably to be 16β-methyl-Δ4-pregnene-9α,11α-epoxy-17α,21-diol-3,20-dione. Its significance either as an intermediate of the dienization or as a by-product is rather uncertain.

The fermentation was carried out as follows: one slant culture of *Corynebacterium simplex* was used to inoculate a 500 ml Erlenmeyer flask containing 100 ml of a liquid medium of the following composition: peptone 0.6%, casein 0.4%, yeast autolysate 0.3%, meat extract 0.15%, glucose 0.1%. After being incubated on a rotary shaker at 200 rpm and at a temperature of 27°C for 18 h, the contents of the flask served as inoculum for 4 l of the same medium in a glass jar. This broth was incubated at 28°C for 14 h under constant agitation at 500 rpm and aeration at a rate of 1 v/v per min. Subsequently 1.5 g of 16β-methyl-5α-Δ9(11)-pregnene-17α,21-diol-3,20-dione-21-acetate dissolved in 40 ml of methanol was added to the broth. The fermentation was continued for another 15 h

[1] D. KLUEPFEL and C. CORONELLI, Exper. *18*, 441 (1962). A printing mistake occurred in this communication: the name of compound I should read 16β-methyl-5α-dihydrocortisone instead of 16β-methyl-5α-dehydrocortisone.

[2] B. M. BLOOM and G. M. SHULL, J. Amer. chem. Soc. *77*, 5767 (1955).

and then the whole culture broth was exhaustively extracted with chloroform. The extract, after having been concentrated to a small volume under reduced pressure, was poured into petroleum ether in order to precipitate the steroid compound. The crude material was purified by column chromatography on florisil and recrystallized from ethyl acetate. The product had the following characteristics: m.p. 196–198°C; $[\alpha]_D + 67.4°$ (dioxane), Anal. calcd. for $C_{22}H_{28}O_5$: C, 70.95; H, 7.58; O, 21.48. Found: C, 70.70; H, 7.66; O, 21.25; λ_{max}^{MeOH} 238 mμ, lg ε = 4.15; IR spectrum (chloroform): 3600 and 3450 cm^{-1} (ν OH), 1702 cm^{-1} (ν C_{20}=O), 1660 cm^{-1} (ν C_3=O), 1620 and 1610 (sh) cm^{-1}) (ν C=C of Δ_{1-4} group), 997 cm^{-1} (considered characteristic for 9α,11α-epoxy configuration), 885 cm^{-1} (CH out of plane deformation of Δ_{1-4} group). For further identification this compound was acetylated in the position 21 with acetic anhydride in pyridine, thus having the following characteristics: m.p. 198–200°C; $[\alpha]_D + 81°$ (dioxane); Anal. calcd. for $C_{24}H_{30}O_6$: C, 69.54; H, 7.29; O, 23.16. Found: C, 69.84; H, 7.52; O, 23.41; λ_{max}^{MeOH} 238 mμ, lg ε = 4.2. This product was identical with a synthetic sample of 16β-methyl-Δ1,4-pregnadiene-9α,11α-epoxy-17α,21-diol-3,20-dione-21-acetate obtained by treatment of 16β-methyl-Δ1,4,9(11)-pregnatriene-17α,21-diol-3,20-dione-21-acetate with perbenzoic acid[3].

The small quantities of a by-product occasionally appearing in some fermentations were revealed by thin layer chromatography on silica gel. In order to isolate this unknown product, the crude material was acetylated in the position 21 and subjected to column chromatography on florisil using chloroform-2% methanol as eluent. The first fractions yielded a substance which was recrystallized from ethyl acetate: m.p. 195–197°C; $[\alpha]_D + 87.9°$ (dioxane), Anal. calcd. for $C_{24}H_{32}O_6$: C, 69.20; H, 7.74; O, 23.05. Found: C, 68.50; H, 7.44; O, 23.65; λ_{max}^{MeOH} 238 mμ, lg ε = 4.1; IR-spectrum (chloroform): 3480 cm^{-1} (ν OH), 1748 cm^{-1} (ν C=O acetate), 1733 cm^{-1} (ν C_{20}=O), 1670 cm^{-1} (ν C_3=O), 1620 cm^{-1} (ν C_4=C_5), 998 cm^{-1} (9α,11α epoxy configuration). The IR-spectrum revealed the presence of the band at 998 cm^{-1} and the absence of the one at 885 cm^{-1}. The compound was different from a synthetic sample of 16β-methyl-Δ1-pregnene-9α,11α-epoxy-17α,21-diol-3,20-dione-21-acetate. From all these data we attributed the double bond to the position 4 and conclude that this by-product is 16β-methyl-Δ4-pregnene-9α,11α-epoxy-17α-21-diol-3,20-dione.

Zusammenfassung. Während unserer Untersuchungen über die mikrobiologische Dehydrierung verschiedener 16β-Methylsteroide durch Bakterienkulturen von *Corynebacterium simplex*, wurde eine neue Art von Umwandlung beobachtet. Von 16β-Methyl-Δ9(11)-pregnen-17α,21-diol-3,20-dion-21-acetat ausgehend, gelang es aus den Kulturfiltraten 16β-Methyl-Δ1,4-pregnadien-9α,11α-epoxy-17α,21-diol-3,20-dion zu isolieren. In einigen Fällen wurde auch das Δ4-Derivat dieser Verbindung als Nebenprodukt erhalten[4].

C. Coronelli, D. Kluepfel, and P. Sensi

Research Laboratories of Lepetit S.p.A., Milano (Italy), December 4, 1963.

[3] J. Fried and E. S. Sabo, J. Amer. chem. Soc. *79*, 1130 (1957).

[4] *Acknowledgments.* The authors are indebted to Dr. C. R. Pasqualucci for the interpretation of IR- and UV-spectra and to Dr. G. G. Nathansohn for supplying synthetic 16β-methyl-5α-Δ9(11)-pregnene-17α,21-diol-3,20-dione-21-acetate.

A Calcifying Topical Reaction to Mast Cell Depletors in the Rat[1]

Subcutaneous injection of certain mineral salts (e.g. lead, cadmium salts) to the rat results in massive local calcification of dermal collagen fibres[2]. Recently, several compounds listed in the Table were found to elicit a different type of local calcification with predominant involvement of striated muscle fibres at their subcutaneous injection site[3] (Figure 1). These calcifying compounds share the additional properties of being complex organic bases, mast cell depletors[4] and antiheparins as well[5]. The muscular calcification is largely dose-dependent and its intensity independent of an occasional cutaneous necrosis. All other tested mast cell depletors (e.g. dextran, histamine, serotonin, albumen, arginine) produced no such calcifying reaction even though some of them be complex bases.

For histogenetic study, female Sprague-Dawley rats (90–100 g body weight), kept on 'Purina Laboratory Chow' and tap water, were subcutaneously injected in the back with a single dose of polymyxin B sulfate (1.5 mg in 0.2 ml of distilled water) and killed in pairs with chloroform inhalation at various intervals thereafter. Skin specimens at injection site were carefully dissected so as to prevent traumatic disruption of the mast cells, immediately fixed in alcohol-formol (4 parts of absolute alcohol and 1 part of neutral formaldehyde) and embedded in paraffin; sections were stained with a von Kóssa-Azure A procedure for the combined demonstration of calcium phosphates and metachromatic material.

1 h after injection with polymyxin, calcium phosphates were demonstrable in most mesenchymal cells (probably 'activated' fibroblasts), first in their cytoplasm then in and around their nuclei (Figure 2A). After 5 h, liberated hypodermal mast cell granules began to calcify. After 24 h, cutaneous muscle (Figure 2B) and subcutaneous nerves (Figure 2C) showed heavy deposition of calcium. The dermal connective fibres were not visibly calcified. All six

[1] This work was supported by the Medical Research Council (Canada) and the National Institute of Neurological Diseases and Blindness, U.S.P.H.S.

[2] H. Selye, *Calciphylaxis* (University Press, Chicago 1962).

[3] J.-M. Dieudonné, Thesis, University of Montreal (1963).

[4] W. D. M. Paton, Pharmacol. Rev. *9*, 269 (1957). – E. T. Kimura, P. R. Young, and R. K. Richards, Proc. Soc. exp. Biol. Med. *107*, 19 (1961).

[5] I. Mota, W. T. Beraldo, and L. C. U. Junqueira, Proc. Soc. exp. Biol. Med. *83*, 455 (1953). – E. T. Kimura, P. R. Young, R. J. Stein, and R. K. Richards, Toxicol. appl. Pharmacol. *1*, 185 (1959).