for the free hydroxy compound (V). With methyllithium in ether, the addition reaction was very rapid (30 min), but the yield (70%) was somewhat diminished. The final step in the projected transformation was completed by Oppenauer oxidation with cyclohexanone and aluminium isopropoxide in the usual manner to furnish methyltestosterone (VIII), m.p. and mixture m.p. 164–166°, ε_{max} 2410 Å = 16,300 (in EtOH), in 87% yield. An interesting modification was discovered during the course of these investigations and consisted in the use of activated alumina in place of the conventional alkoxide catalyst. Thus, the diol (VI), on treatment with cyclohexanone and alumina (3 g/g steroid) at the reflux temperature of toluene for 1 h was smoothly converted into methyltestosterone (VIII) in 80% yield, taking into consideration a 17% recovery of starting material. The heterogeneous oxidation, though closely related to, is mechanistically different from the Oppenauer reaction, which involves a quasi six-membered cyclic transition state[9], depicted in (IX). It is unlikely that such an activated complex obtains with the alumina catalysed oxidation, which presumably entails co-adsorption of the diol (VI) and cyclohexanone on the active surface of the catalyst with subsequent hydrogen transfers from the steroid-donor to the ketone-acceptor. In this respect, the reaction is reminiscent, in general, of the heterogeneously catalysed transferhydrogenations, studied extensively by BRAUDE, LINSTEAD *et al.*[10], and in particular, of the Raney nickel catalysed oxidations[11] with cyclohexanone.

In an alternative route to the diol (VI) from the dihydroxy-ketone (IVa), the sequence of reactions was reversed, i.e., the C_{17} side-chain was elaborated preparatory to the construction of the 3β-hydroxy-Δ^5-grouping. Treatment of (IVa) with methylmagnesium chloride in tetrahydrofuran under conditions identical to those used with (V), surprisingly gave a comparatively low yield (45%) of 17α-methyl-3α,6α,17β-aetiocholantriol (VIIa), m.p. 230–232°, $[\alpha]_D^{26}$ −23·47 (*c*, 0·649) (Found: C, 74·27; H, 10·61; O, 15·10%. $C_{20}H_{34}O_3$ requires C, 74·49; H, 10·63; O, 14·89%). *A priori*, this was ascribed to the poor solubility of (IVa) in the reaction medium, particularly as some (12%) recovery of the starting material was made. However, this could hardly have been the reason, since replacement of (IVa) by its diacetate (IVb), which is quite soluble in tetrahydrofuran and gives a soluble Grignard complex, still failed to improve the yield. Tosylation of the triol (VIIa) with pyridine and tosyl chloride at 0–5°, and subsequent dehydrotosylation of the resultant ditosylate (VIIb), m.p. 145° decomp., $[\alpha]_D^{26}$ − 10·74 (*c*, 0·849) (Found: C, 64·70; H, 7·49; O, 17·98; S, 10·22%. $C_{34}H_{46}O_7S_2$ requires C, 64·73; H, 7·35; O, 17·75; S, 10·16%), as described above with (IVc), furnished 17α-methyl-3β,17β-Δ^5-androstenediol (VI), m.p. and mixture m.p. 197–201°, $[\alpha]_D^{30}$ − 74·2 (*c*, 1·105, alcohol) in 45% yield. The isolation of pure (VI) was a matter of considerable difficulty and was best achieved by conversion to, and regeneration from its oxalic acid adduct[12].

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[10] E. A. BRAUDE and R. P. LINSTEAD, J. chem. Soc. *1954*, 3544, and subsequent papers in this series.

[11] E. C. KLEIDERER and E. C. KORNFELD, J. org. Chem. *13*, 455 (1948).

[12] K. MIESCHER and H. KÄGI, Helv. chim. Acta *24*, 986 (1941). – L. YODER, U.S.Patent, 2,362,605 (1944).

Full details of the work will be published at a later date, elsewhere.

K. R. BHARUCHA

Research Laboratories, Canada Packers Limited, Toronto, July 29, 1957.

Zusammenfassung

Der Hauptbestandteil der Gallenflüssigkeit des Schweines, Hyodesoxycholsäure, wurde in die männlichen Geschlechtshormone Testosteron und Methyltestosteron überführt. Es wird eine modifizierte Oppenauer-Oxydation beschrieben, bei der Aluminiumoxyd an Stelle des gebräuchlichen Alkoxyd-Katalysators verwendet wird.

On the Role of the 4-Formyl Group of the Pyridoxal-5-phosphate in the Activation of Apotransaminase

In a paper by COHEN[1], the inhibition of transaminase activity by cyanide ions was ascribed to their action on the oxalacetic (or pyruvic) acid resulting in cyanohydrin formation. This assumption on the inhibition mechanism was never modified even when pyridoxal-5-phosphate (Py-5-P) was recognized as the coenzyme of transaminase reactions.

In the attempt to demonstrate an alternative inhibition mechanism characterized by cyanohydrin formation due to the reaction between CN-ions and the 4-formyl group of Py-5-P, we have followed the transaminase reaction (*a*) after addition to the activated system of KCN, and (*b*) after addition to apotransaminase of Py-5-P previously incubated with KCN.

Our results do not appear to be in agreement with the accepted role played by 4-formyl group of Py-5-P in the scheme of SCHLENK and FISHER[2], suggesting a particular role of this group in the attachment of the coenzyme to apotransaminase.

The glutamic-oxalacetic transaminase (GOT), used in our experiments, was prepared from pig heart as described by O'KANE and GUNSALUS[3], and purified up to the stage of heating at 60°C; the resolution was of about 95% and specific activity of 3·4 (μ*M* of oxalacetic acid formed by 1 mg protein per minute).

The formation of oxalacetic acid was followed at 280 mμ in a Beckman spectrophotometer mod. DU, equipped with thermospacer kept at 37°C. All other conditions of transamination reaction were those proposed by CAMMARATA and COHEN[4].

The reaction between Py-5-P and KCN was allowed to occur in stoichiometric amounts of Py-5-P (Hoffmann-La Roche) and KCN in phosphate buffer 0·05 *M*, pH 7·4, for 90 min at 50°C. This addition reaction can be followed spectrophotometrically by the decrease of extinction at 385 mμ (Fig. 1). The velocity constant is 4·65 liter $mole^{-1}$ sec^{-1} at 37°C. At 50°C the reaction is obviously faster and attains the completeness in 90 min. Full details regarding the kinetics of the above reaction will be reported elsewhere.

[1] P. P. COHEN, Biochem. J. *33*, 1478 (1939).

[2] F. SCHLENK and A. FISHER, Arch. Biochem. *12*, 69 (1947).

[3] D. E. O'KANE and I. C. GUNSALUS, J. biol. Chem. *170*, 425 (1947).

[4] P. S. CAMMARATA and P. P. COHEN, J. biol. Chem. *193*, 53 (1951).

(*a*) When GOT, previously incubated for 10 min at 37°C with the optimal amount of Py-5-P (Fig. 2), is reincubated with KCN in large excess (10^{-2} *M*), for 25 min at 37°C, in phosphate buffer 0·05 *M*, pH 7·4, and subsequently dialysed for 12 h against the same buffer, the enzymatic system shows the same activity as that shown in similar experiments without KCN, simultaneously run.

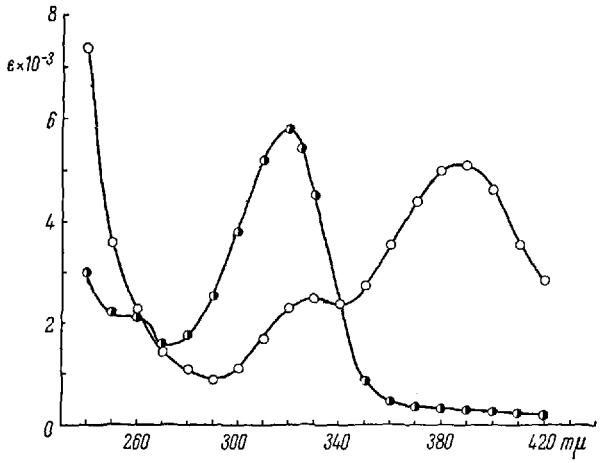

Fig. 1. – Absorption spectra at pH 7·4: o–o, pyridoxal-5-phosphate; •–•, pyridoxal-5-phosphate after reaction with equivalent KCN.

(*b*) The Py-5-P, after reaction with KCN in stoichiometric amounts, fails to activate the apotransaminase. Further, its addition even in seven fold excess (either simultaneously or after Py-5-P) to the resolved GOT does not modify the activation effect due to increasing amounts of Py-5-P, at least in our experimental conditions.

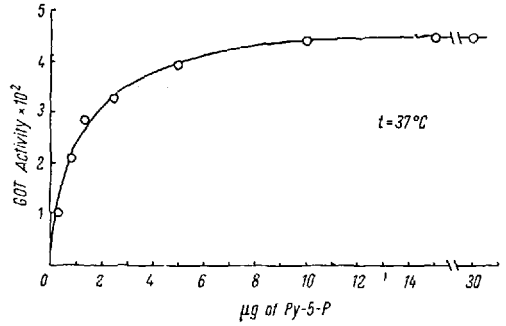

Fig. 2. – GOT activity as a function of pyridoxal-5-phosphate added to the incubation mixture containing 1 ml of the 1:20 enzyme solution. GOT activity × 10^2 equal to one corresponds to 0·034 μMoles oxaloacetate formed by 1 mg protein per minute.

A role of 4-formyl group quite different from that suggested by SCHLENK and FISHER[2] may be inferred from our results. It seems, indeed, that the 4-formyl group must be involved in the attachment of the coenzyme to apotransaminase rather than in the formation of a Schiff base with aminoacid substrate.

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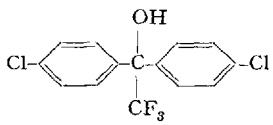
Riassunto

Vengono descritte indagini sull'interazione tra piridossal-5-fosfato e KCN. Il composto di addizione non attiva l'apotransaminasi, ma neppure compete con il piridossal-5-fosfato.

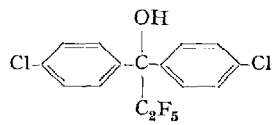
I risultati lasciano intravedere che il gruppo carbonilico di quest'ultimo sia interessato nell'attacco del coenzima stesso all'apoenzima.

Reduced Oviposition in *Aedes aegypti* L. Following Tarsal Exposure to a Fluorocarbon[1]

It has been shown recently[2] that di-(*p*-chlorophenyl)-trifluoromethylcarbinol (I) and di-(*p*-chlorophenyl)-pentafluoroethylcarbinol (II) synthesized by BERGMANN *et al.*[3] reduce substantially or even inhibit completely oviposition in the housefly upon tarsal contact.



I



II

Although II was pronouncedly better as an 'O.I.T.C.-agent' in houseflies on continuous exposure[2] than I, in the present work on *Aedes aegypti*, the more readily available I was employed. It was soon found that mosquitoes kept in continuous contact with deposits of I showed even at very low concentrations either an enhanced mortality due to the slight toxicity of the compound[4], or refused to take their blood meals. Therefore, tarsal contact of short duration followed by a single blood meal was found to be the method of choice.

The number of eggs deposited by *Aedes aegypti* after a single blood meal depends on sufficient larval diet[5], on fertilization of the females[6] and on the amount of blood ingested[7]. Accordingly, 4–5 days old females were taken out from mixed population cages[8] of *Aedes aegypti* bred from well-fed larvae and kept at 28°C, 80% R.H. on 10% glucose. The females were exposed at 24°, under an inverted glass funnel to a deposit of 1 g/sq.m. I on filter paper, exposure times being 10, 20, 30 and 40 min. After the exposure, they were returned to 28°, starved for 20–24 h and then were allowed to feed for 3 h on 'Nembutal'-immobilized guinea pigs. All engorged females

[1] The abbreviation O.I.T.C.-agents (oviposition-inhibiting tarsal contact agents) is suggested for compounds reducing or inhibiting oviposition in insects upon tarsal contact.

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[8] D. R. SEATON and W. H. R. LUMSDEN, Ann. trop. Med. Parasit. *35*, 23 (1941), found that 82% of females in cages had mated 72 h after emergence.