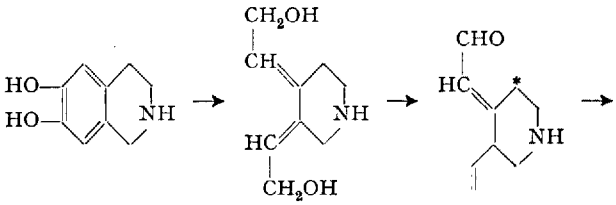
The qualitative aspect of the experimental results available in November 1957, which show the violation of the C- and the P-invariance for weak interactions, is reviewed. The methods hereby applied are betadecay of oriented nuclei, polarisation of emitted electrons in beta-decay, beta-gamma-correlation, asymmetry in the decay of μ-mesons generated by π-meson-decay. The solution of the Θ-τ-puzzle by the assumption of a single particle (K-meson) without defined parity is mentioned.

In the concluding section, some aspects of the unsolved theoretical problems of the deeper reasons for the symmetry violations of the weak interactions are briefly discussed which will possibly also lead into open cosmological questions.

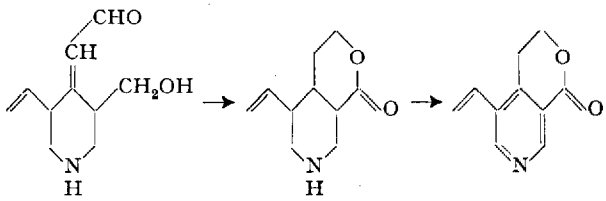
Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

A Biogenetic Scheme for Gentianine

The alkaloid gentianine to which structure (I) has been assigned on the basis of degradation[1] and synthetic evidence[2], is unique in having carbon substituents at the 3-, 4-, and 5-positions of the pyridine ring. A plausible biosynthetic route to this alkaloid is suggested by the presence of two 2-carbon fragments in adjacent positions. WOODWARD fission[3] of 1:2:3:4-tetrahydro-6:7-dihydroxyisoquinoline (II) would yield the pyridine (III)



(II) (III) (IV)



(V) (VI) (I)

which can give by oxidation-reduction and dehydration, the vinyl compound (IV). Attack at the allylic (starred) position in (IV) by formaldehyde or equivalent[4] would give the alcohol (V), which through oxidation-reduction to the lactone (VI) and dehydrogenation would yield gentianine (I). The sequence of steps outlined here need not be the same in the plant, but it is of interest that gentianine furnishes the simplest example of an alkaloid whose formation is best explained by invoking a WOODWARD fission.

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K. NAGARAJAN, and S. RAJAPPA

Department of Chemistry, Presidency College, Madras (India), August 13, 1957.

Zusammenfassung

Für die Biogenese des Gentianins wird ein plausibles Reaktionsschema vorgeschlagen.

[1] T. R. GOVINDACHARI, K. NAGARAJAN, and S. RAJAPPA, J. chem. Soc. *1957*, 551. – N. F. PROSKURNINA, J. gen. Chem., Moscow, *14*, 1148 (1944). – N. F. PROSKURNINA, V. V. SHPANOV, and R. A. KONOVALOVA, Doklady Akad. Nauk SSSR. *66*, 437 (1949). – N. F. PROSKURNINA and V. V. SHPANOV, Zh. Obshch. Khim. *26*, 936 (1956).

[2] T. R. GOVINDACHARI, K. NAGARAJAN, and S. RAJAPPA, J. chem. Soc. *1957*, 2725.

[3] R. B. WOODWARD, Nature *162*, 155 (1948).

[4] A. LAPWORTH, J. chem. Soc. *79*, 1276 (1901). – W. BORSCHE and R. MANTEUFFEL, Lieb. Ann. *505*, 177 (1933). – R. ROBINSON, *The Structural Relations of Natural Products* (Oxford, 1955), p. 15.

Testosterone and Methyltestosterone from Hyodesoxycholic Acid

A recent report[1] from these Laboratories described the conversion of hyodesoxycholic acid (3α, 6α-dihydroxycholanic acid) (I), the main constituent of hog-bile into the corpus luteum hormone – progesterone. The present communication is a logical extension of these studies to include the preparation of analogous male sex hormones – testosterone and its synthetic homologue, methyltestosterone (VIII).

The diacetate of 3α, 6α-dihydroxypregnan-20-one (II), readily obtained[1] from the bile acid (I) in 55% yield by the Meystre-Miescher degradation, was enol-acetylated in carbon tetrachloride solution with acetic anhydride and a trace of perchloric acid (70–72%)[2]. In view of the geometrical isomers, anticipated on the formation of the Δ^{17}-double bond[3], no attempt was made to isolate the triacetate (III), which was directly

[1] K. R. BHARUCHA, G. C. BUCKLEY, C. K. CROSS, L. J. RUBIN, and P. ZIEGLER, Can. J. Chem. *34*, 982 (1956).

[2] D. H. R. BARTON, R. M. EVANS, J. C. HAMLET, P. G. JONES, and T. WALKER, J. chem. Soc. *1954*, 747.

[3] C. W. MARSHALL, T. H. KRITCHEVSKY, S. LIEBERMAN, and T. F. GALLAGHER, J. Amer. chem. Soc. *70*, 1837 (1948). – L. F. FIESER and HUANG-MINLON, J. Amer. chem. Soc. *71*, 1840 (1949).

ozonised *in situ* in a mixture (1:1) of carbon tetrachloride and aqueous acetic acid (95% v/v). Reductive decomposition of the ozonide with zinc dust, followed by saponification, gave 3α,6α-dihydroxyaetiocholan-17-one (IVa), m.p. 246–250°, $[\alpha]_D^{29}$ + 52·65 (*c*, 1·675[4]) (Found: C, 74·46; H, 9·97; O, 15·63%. $C_{19}H_{30}O_3$ requires C, 74·47; H, 9·87; O, 15·66%), characterised as its diacetate (IVb), m.p. 141–143°, $[\alpha]_D^{26}$ + 65·28 (*c*, 1·034) (Found: C, 70·58; H, 8·70; O, 20·7%. $C_{23}H_{34}O_5$ requires C, 70·76; H, 8·71; O, 20·51%). The conversion of 3α,6α-dihydroxy groups of (IVa) into 3β-hydroxy-Δ^5-moiety, a precursor of 3 keto-Δ^4-grouping was effected following the method developed earlier in these Laboratories[5]. The ditosylate (IVc), m.p. 158–159° (decomp.), $[\alpha]_D^{27}$ + 40·03 (*c*, 1·768) (Found: C, 64·63; 64·62; H, 6·92; 6·95; O, 18·24; S, 10·42%. $C_{33}H_{42}O_7S_2$ requires C, 64·47; H, 6·89; O, 18·22; S, 10·43%), derived from the diol (IVa) in near quantitative yield, by treatment with pyridine and *p*-toluenesulphonyl chloride, was heated with potassium acetate in aqueous dimethylformamide at 100° for 4½ h. Saponification and chromatographic purification then afforded the known Δ^5-dehydroepiandrosterone (V) (34% overall yield from II), m.p. and mixture m.p. 148–151°, convertible to testosterone by well-established methods[6].

The synthesis of the higher homologue – methyltestosterone (VIII) from (V) was accomplished via Grignard and Oppenauer reactions. Addition of methylmagnesium iodide in ether to the ketone (V) was initially reported by RUZICKA *et al.*[7], to give 17α-methyl-3β,17β-Δ^5-androstenediol (VI), the yield (57%) being subsequently improved[8] to 74% by the simple expedient of using a ten-fold excess of the Grignard reagent. In the present work, methylmagnesium chloride, readily prepared in 90% yield, in tetrahydrofuran, was utilised and afforded the 17α-methyl-diol (VI), m.p. and mixture m.p. 199–202°, in *ca.* 80% yield. Similar result was obtained, when dehydroepiandrosterone acetate was substituted

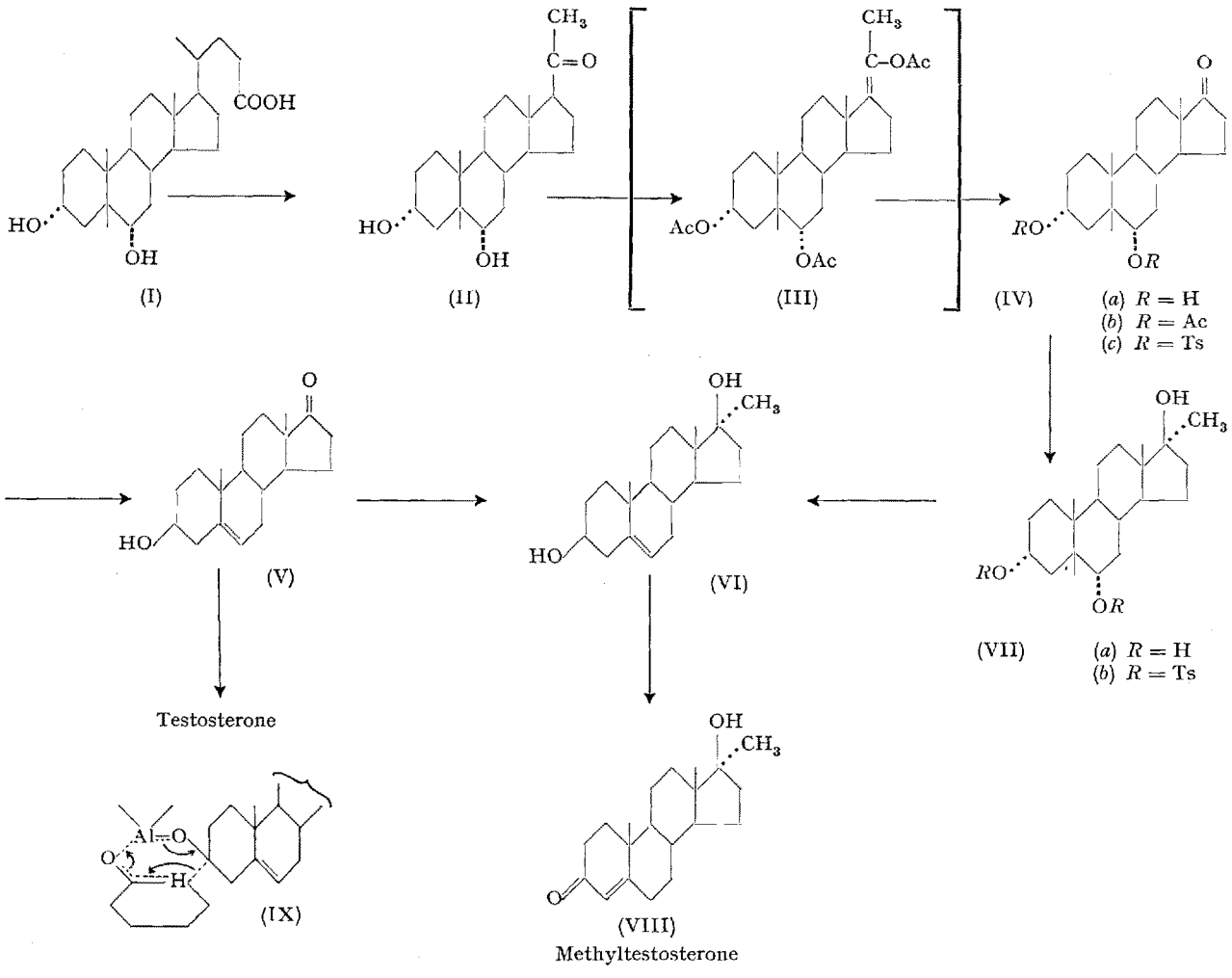
[4] All rotations were measured in a 1 dm tube, in dioxane, unless otherwise stated.

[5] K. R. BHARUCHA, G. C. BUCKLEY, C. K. CROSS, L. J. RUBIN, and P. ZIEGLER, Can. J. Chem. *34*, 982 (1956). – P. ZIEGLER and K. R. BHARUCHA, Chem. and Ind. *1955*, 1351. – See also L. VARGHA and M. RADOS, Chem. and Ind. *1955*, 896. – L. VARGHA, M. RADOS, and M. KRAUT, Acta chim. Acad. Sci. Hung. *8*, 303 (1955). – S. BERGSTROM and K. PAABO, Acta chem. Scand. *9*, 699 (1955).

[6] *Inter alia:* L. F. FIESER and M. FIESER, *Natural Products Related to Phenanthrene* (Reinhold Publishing Corp., New York 1949), p. 371. – A. C. OTT, M. F. MURRAY, and R. L. PEDERSON, J. Amer. chem. Soc. *74*, 1239 (1952). – F. SONDHEIMER, C. AMENDOLLA, and G. ROSENKRANZ, J. Amer. chem. Soc. *75*, 5930 (1953). – H. J. DAUBEN, Jr., B. LÖKEN, and H. J. RINGOLD, J. Amer. chem. Soc. *76*, 1359 (1954).

[7] L. RUZICKA, M. W. GOLDBERG, and H. R. ROSENBERG, Helv. chim. Acta *18*, 1487 (1935).

[8] G. I. KIPRIANOV and B. E. FRENKEL, J. gen. Chem., Moscow *9*, 1682 (1939); Chem. Abstr. *34*, 3756 (1940).



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].

[9] R. B. WOODWARD, N. L. WENDLER, and F. J. BRUTSCHY, J. Amer. chem. Soc. *67*, 1428 (1945). – L. M. JACKMAN and A. K. MACBETH, J. chem. Soc. *1952*, 3252. – W. VON E. DOERING and T. C. ASCHNER, J. Amer. chem. Soc. *75*, 393 (1953).

[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).