Characterization of 17-Oxosteroids by Derivatives formed in the Zimmermann Reaction

DURING a study of 17-oxosteroid levels in blood plasma it was found that the amount of individual compounds available was frequently too small to permit determination and also identification by paper chromatography. After the determination of isolated 17-oxosteroids by Zimmermann reaction it is possible to recover these compounds as derivatives which can be used for characterizing the compounds measured.

The solution from the colorimeter cell was diluted with eight volumes of water and one volume of concentrated hydrochloric acid added. The acidified solution, which is colourless, was extracted with benzene ($3 \times \frac{1}{2}$ vol.) and the combined benzene extracts washed with water until neutral. When the Zimmermann reaction was repeated on the material extracted by benzene, the extinction corresponded to 60–95 per cent recovery of steroid; the colour appeared immediately with alkali alone and maximum intensity was obtained under Zimmermann conditions in 20 min.

When the recovered Zimmermann complexes of the separated 17-oxosteroids (approximately 5 μ gm.) were chromatographed on No. 2 Whatman paper in a hydrocarbon/aqueous methanol system¹ spots could be located as dark areas by viewing in ultra-violet light of maximum intensity in the 240 m μ region (Hanovia OX7 filter). These areas were also revealed as purple spots by dipping in ethanolic potassium hydroxide (10 per cent w/v). The immediate appearance of the purple colour together with the absorption of ultra-violet light suggests that the *m*-dinitrobenzene is combined with the steroid on the paper chromatogram. These complexes appear to be very stable as they were not destroyed by hot acid hydrolysis (10 per cent hydrochloric acid v/v at 100° for 15 min.) or by the subsequent washing of benzene extracts by alkali.

Table 1, which gives the R_F values of the free 17-oxosteroids and of their derivatives when run in Bush partition systems, shows that whereas in the *A* system the free compounds run faster than their derivatives, in the *B*₁ system they run more slowly. This diverse behaviour appears to be associated with the partition system used and not with the C₁₁-oxygen function, for the 11-oxo-aetiocholanolone derivative runs in the *A* system more slowly than the corresponding free compound.

Table 1. R_F VALUES OF STEROIDS AND DERIVATIVES ON WHATMAN NO. 2 PAPER
(Equilibration time, 3 hr.; running time, 4 hr.; temperature, 37°)

	Free steroid	Derivative
Bush <i>A</i> system		
Dehydro Δ^4 androsterone	0.32	0.23
Δ^4 Androsterone	0.37	0.31
Aetiocholanolone	0.43	0.33
Androsterone	0.56	0.46
11-Oxo-aetiocholanolone	0.064	0.042
Modified Bush <i>B</i>₁ system		
11-Hydroxy-aetiocholanolone	0.40	0.70
11-Hydroxy-androsterone	0.50	0.78
11-Oxo-aetiocholanolone	0.64	0.83
11-Oxo-androsterone	0.70	0.88

Bush *A* system: 80–100° petroleum ether (10), methanol (8), water (2).
Modified Bush *B*₁ system: 80–100° petroleum ether (5), benzene (5), methanol (7), water (3).
 R_F values calculated against sudan IV, which was used for indicating the solvent front approximately.

The Zimmermann complexes formed from 5 α -androst-3:17-dione, 5 β -androst-3:17-dione, androst-4-ene-3:17-dione and androst-4-ene-3:11:17-trione did not move from the origin in Bush *A*, *B*₁ or *B*₂ systems.

This technique has enabled us to obtain further evidence of identity of steroids isolated from blood plasma by chromatography on alumina.

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¹ Bush, I. E., *Biochem. J.*, **50**, 370 (1952).

An Asymmetric Catalyst

ASYMMETRIC synthesis has hitherto succeeded only by using reagents or solvents having the asymmetric configuration.

We have been able to synthesize optically active amines and amino-acids by using an asymmetric catalyst, based on the fact that the optical activity of the biological substances originates in the asymmetric structure of the enzyme proteins.

The asymmetric catalyst was obtained by reduction of protein-palladium complex which was prepared by adsorption of palladium chloride on silk fibroin fibre.

Optically active glutamic acid, phenylalanine and diphenylethylenediamine were formed by hydrogenating their precursors, diethyl α -acetoximinoglutarate, ethyl α -acetoximinophenyl-propionate, 4-benzilidene-2-methyloxazol-5-one and α -benzildioxime, respectively, in the presence of this catalyst.

Preparation of catalyst. 100 mgm. of palladium chloride was adsorbed on 300 mgm. of silk fibroin fibre, and the complex was reduced with hydrogen in 50 ml. methanol at 50° C., 80 kgm./cm.² for 1 hr.

Hydrogenation of diethyl α -acetoximinoglutarate. 10 gm. of diethyl α -acetoximinoglutarate was hydrogenated with the catalyst in 50 ml. methanol at 50° C., 80 kgm./cm.². The reaction mixture was filtered, evaporated *in vacuo*, hydrolysed for 1 hr., precipitated with hydrochloric acid, and extracted with acetone to remove organic acids. Then the product was recrystallized four times. Glutamic acid was obtained in a yield of 39 per cent. $[\alpha]_D^{15} = +2.25^\circ$ (c., 7 per cent in 2N HCl). Analysis: calculated for C₅H₁₀O₄NCl: N, 7.63; found: N, 7.36.

This compound was identified by paper chromatography and infra-red spectroscopy. The manometric analysis (by glutamic acid decarboxylase of *E. coli*) showed the same value of L(+) content.

Hydrogenation of ethyl- α -acetoximinophenyl-propionate. 6 gm. of ethyl- α -acetoximinophenyl-propionate was hydrogenated with the catalyst in 50 ml. methanol at 70° C., 90 kgm./cm.². The reaction mixture was filtered, evaporated *in vacuo*, hydro-