Full details of this and further work will be published in *Helvetica Chimica Acta*.[14]

(14) Drs. E. J. Corey and W. R. Hertler also have been concerned with this problem and our results are published simultaneously with theirs by friendly agreement.

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RECEIVED MAY 1, 1958

ACYL AMIDES AS EPIMERIZATION REAGENTS[1,2]

Sir:

We wish to report the novel epimerizing action of acyl amides on certain tosylates.

β-Cholestanyl tosylate in a 2.5% solution in N,N-dimethylformamide[3] (DMF) heated at 78°, reacted completely in 23 hours to form a product which when chromatographed on Florisil[4] was cleanly separated into 75% of α-cholestanyl formate (m.p. 114.5–116.0°, $[\alpha]_D$ +30.3° chf, $\lambda_{max}^{CS_2}$ 5.82; 8.40, 8.45, 8.65μ.[5] *Anal.* Found: C, 81.00; H, 11.80), identical with the ester prepared from epicholestanol[1] and formic acid, and 22% of 2-cholestene.[6] However, chromatography on alumina[7] instead of Florisil resulted in hydrolysis of the formate, permitting a facile quantitative separation[8] into α-cholestanol and olefin.

In DMF purified[9] by treatment with barium oxide and distillation, the reaction, although slower (60 hr.), gave essentially the same yields of products. At reflux temperature, with untreated DMF, the product contained 35% formate and was predominantly olefin.

Methyl 3α-tosyloxycholanate with untreated DMF at 78° gave when chromatographed on Florisil 78% methyl 3β-formoxycholanate (m.p. 104–107.5°, $[\alpha]_D$ +13.4° chf., $\lambda_{max}^{CS_2}$ 5.77, 5.82; 8.45, 8.68μ. *Anal.* Found: C, 74.90; H, 10.27) identical with the product of esterification of methyl 3β-hydroxycholanate[10] with formic acid, and 20% methyl 3-cholenate. Chromatography on alumina similarly yielded methyl 3β-hydroxycholanate and olefin quantitatively.

Obtained at 78° from DMF and the corresponding epimeric tosylates were: β-cholestanyl formate,[11] 45 hr., 36%, m.p. 85–86.0°, $[\alpha]_D$ + 14.4° chf, $\lambda_{max}^{CS_2}$ 5.82; 8.47μ (*Anal.* Found: C, 80.91; H, 11.85); androsterone formate, 45 hr., 73%, m.p. 181–181.5°, $[\alpha]_D$ +94.2° chf, $\lambda_{max}^{CS_2}$ 5.76, 5.82; 8.44, 8.65μ. (*Anal.* found: C, 75.25; H, 9.60). Cholesteryl tosylate under these conditions in 40 hr. gave 54% of cholesteryl formate,[12] m.p. 97.5–98.0°, $[\alpha]_D$ −49.1° chf, $\lambda_{max}^{CS_2}$ 5.81; 8.45, 8.52μ.

With N,N-dimethylacetamide,[3] β-cholestanyl tosylate required 92 hr. for complete reaction (disappearance of strong tosylate infrared bands), yielding 21% of α-cholestanyl acetate,[13] and much ether-insoluble product.

Formamide did not react with β-cholestanyl tosylate because of the extremely low solubility, but when heated for 65 hr. at 78° with methyl 3α-tosyloxycholanate, formed 53% of methyl 3β-hydroxycholanate and 32% of methyl 3-cholenate.

Observed facts pertinent to a study of the mechanisms of the reactions reported are: the alcoholic products are stereochemically nearly homogeneous; the reaction in formamide yields inverted alcohol, not formate; purified DMF gives the same yields as untreated DMF, albeit more slowly; an equimolar amount of dimethylammonium *p*-toluenesulfonate was recovered (from DMF reaction); DMF with added *p*-toluenesulfonic acid does not formylate epicholestanol to any appreciable extent under the conditions of the inversion; formamide does not hydrolyze methyl 3β-formoxycholanate under like conditions; cholesterol is formylated without rearrangement.[14]

Full details and further work on this reaction will be reported subsequently.

(1) Paper VII in Seroflocculating Steroids series. Previous paper VI, *Chem. & Ind.*, in press (1958).

(2) This work is supported by grants CS-9053, C-2249 and C-3407 from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

(3) Eastman Kodak Co. product, "Eastman" grade, used as purchased.

(4) Floridin Co. product, 60–100 mesh.

(5) The very strong carbonyl band at 5.82 μ permits estimation of the formate concentration. Such estimates were found to check actual isolations by chromatography to within 1%. Furthermore, the C—O stretching bands near 8.5 μ appear to be characteristic of the 3-formates (L. J. Bellamy, "The Infra-Red Spectra of Complex Molecules," Methuen & Co. Ltd., London, 1956, p. 161).

(6) Elsevier's "Encyclopaedia of Organic Chemistry," Vol. III, 14 Supplement, 1422S.

(7) Fisher Scientific Co. product A-540.

(8) In another experiment in which β-cholestanyl tosylate was heated for 29 hr. at 78° as a 5% solution in DMF, some unreacted tosylate remained, and 3.5% of β-cholestanol was obtained as the final fraction from the chromatography on alumina.

(9) *Cf.* G. R. Leader and J. F. Gorley, THIS JOURNAL, **73**, 5731 (1951). S. R. Ross and M. M. Labes (*ibid.*, **79**, 4155 (1957)), report that DMF purified similarly contains 0.09–0.13% water, more than the equimolar proportions needed for reaction.

(10) R. T. Blickenstaff and F. C. Chang, *ibid.*, **80**, 2726 (1958).

(11) Elsevier's "Encyclopaedia of Organic Chemistry," Vol. III. 14, p. 58.

(12) Elsevier's "Encyclopaedia of Organic Chemistry," Vol. III, 14, p. 1630S.

(13) C. W. Shoppee, *J. Chem. Soc.*, 1138 (1946).

(14) *Cf.* S. Winstein and R. Adams, THIS JOURNAL, **70**, 838 (1948).

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RECEIVED MARCH 28, 1958

THE REACTION OF SODIUM BOROHYDRIDE WITH MUSCLE PHOSPHORYLASE[1]

Sir:

The presence of a firmly bound, non-protein constituent in skeletal muscle phosphorylase has been investigated independently in two different laboratories.[2,3,4] Identification of this material as pyridoxal 5′-phosphate (PLP) was first reported by Baranowski, *et al.*[2] Cori and Illingworth[3] found

(1) Supported by the Institutional Grant to the University of Washington by the American Cancer Society, the Initiative 171 Fund of the State of Washington, and the United States Public Health Service (Grant No. A-850).

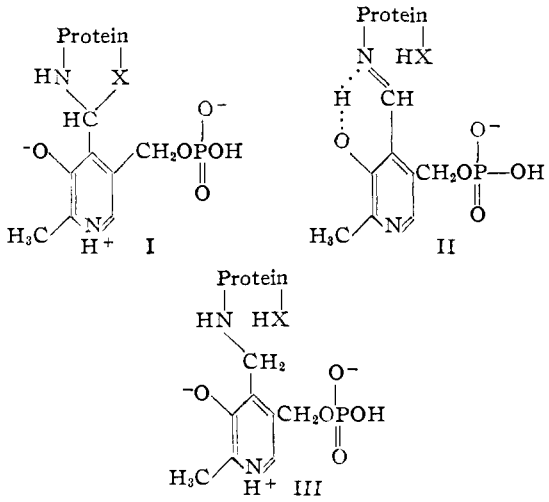
(2) T. Baranowski, B. Illingworth, D. H. Brown and C. F. Cori, *Biochim. et Biophys. Acta*, **25**, 16 (1957).

(3) C. F. Cori and B. Illingworth, *Proc. Nat. Acad. Sci.*, **43**, 517 (1957).

(4) A. B. Kent, E. G. Krebs and E. H. Fischer, *J. Biol. Chem.*, in press.

that the enzyme could be freed of PLP with concomitant loss of activity, which could be restored by readdition of the B_6 derivative.

A study of the spectral properties of phosphorylase[4] has indicated that PLP is bound to the enzyme as a substituted aldamine derivative[5] (I) which is converted to a free Schiff base form (II), and eventually split off the enzyme, by treatment with acid, base or urea.



Further evidence in support of structures I and II has been obtained in a study of the reaction of $NaBH_4$ with phosphorylase, which was undertaken with the hope that (II) could be reduced to a stable pyridoxylamine derivative[6] (III) so that the structure of the active site of the enzyme might be investigated.

Treatment of a solution of the enzyme with $NaBH_4$ at *p*H values where the yellow form II is predominant resulted in immediate decolorization, and the B_6 derivative could no longer be liberated from the protein by acid or base. This reaction was not observed between *p*H 5 and 9.5, where the enzyme shows the spectral properties of form I. To obtain a soluble, fully reduced enzyme, crystalline phosphorylase *b* at 0° was precipitated at one third saturation of ammonium sulfate; the *p*H was brought to 4.5 and $NaBH_4$ was added to a final concentration of 0.5 mg./ml. After centrifugation the protein was dissolved in a neutral buffer and dialyzed.

The reduced enzyme showed an absorption maximum at 330 mμ and gave a positive dichloroquinone-chloroimide test.[7] Surprisingly, it was fully active in the phosphorylase reaction, in the conversion of phosphorylase *b* to *a* catalyzed by phosphorylase kinase, and in the reconversion to *b* catalyzed by PR-enzyme. The possibility of a reoxidation of form III back to form I in the activity test was ruled out.

The reduced enzyme was degraded by chymotrypsin and the pyridoxylamine derivative obtained as a pure peptide by column chromatography,[8] high-voltage electrophoresis and paper chromatography. After acid hydrolysis, a fluorescent, positively charged, ninhydrin reacting compound was isolated and compared to pure synthetic pyridoxyl amino acids, including mono α-, mono ϵ-, and di-pyridoxyllysine. On the basis of its quantitative reactions with ninhydrin and dichloroquinone-chloroimide, its behavior on paper chromatography and its characteristic migration in high voltage paper electrophoresis at *p*H 9.6, it was identified as ϵ-N-pyridoxyllysine.

The finding that the bound PLP of phosphorylase can be reduced to form a stable pyridoxylamine derivative without impairing the enzymatic properties of the enzyme poses a serious question as to the role of the B_6 derivative in the phorphorylase system. This problem is being further investigated, together with the action of $NaBH_4$ on other pyridoxal phosphate dependent enzymes.

(5) H. N. Christensen, THIS JOURNAL, **80**, 99 (1958), has assumed the formation of carbinolamine derivatives in the reaction of PLP with peptides and proteins.

(6) D. Heyl, S. A. Harris and K. Folkers, *ibid.*, **70**, 3429 (1948).

(7) M. Hochberg, D. Melnick and B. L. Oser, *J. Biol. Chem.*, **155**, 109 (1944).

(8) C. H. W. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **219**, 623 (1956).

(9) Public Health Service Research Fellow of the National Heart Institute.

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RECEIVED APRIL 5, 1958

MICROWAVE EXCITATION AS A SYNTHETIC TOOL: THE PREPARATION OF DIBORON TETRACHLORIDE[1]

Sir:

Despite numerous efforts to find more efficient methods for the preparation of diboron tetrachloride (B_2Cl_4), *e.g.*, by the reaction of metal borides with chlorine,[2] by the reduction of boron trichloride with metals, metal borides and other reducing agents,[3] none have been as satisfactory as the electrical discharge method first reported by Stock, Brandt and Fischer[4] and improved by Wartik, Moore and Schlesinger.[5]

Comparable yields have now been obtained by microwave excitation[6] of gaseous boron trichloride at less than 4 mm. pressure. The boron trichloride, maintained at $-78.5°$ is transferred with pumping through the microwave cavity into a $-111.9°$ trap. The free chlorine produced was continuously removed from the $-111.9°$ trap, along with small amounts of boron trichloride and collected in a liquid nitrogen trap.

The diboron tetrachloride retained in the $-111.9°$ trap was purified by fractional distillation of the boron trichloride at $-78.5°$. The diboron tetrachloride was identified by its vapor tension and by infrared spectrum.[7] The determination of the

(1) This work was performed under the auspices of the U. S. Atomic Energy Commission.

(2) E. Apple, Ph.D. Thesis, The Pennsylvania State University, 1955.

(3) G. Urry, T. Wartik, R. E. Moore and H. I. Schlesinger, THIS JOURNAL, **76**, 5293 (1954).

(4) A. Stock, A. Brandt and H. Fischer, *Ber.*, **58**, 855 (1925).

(5) T. Wartik, R. Moore and H. I. Schlesinger, THIS JOURNAL, **71**, 3265 (1949).

(6) Baird Associates' Mercury 198 Exciter operating at a wavelength of 12.2 cm. in the 2400–2500 megacycle band.

(7) M. J. Linevsky, E. R. Shull, D. E. Mann and T. Wartik, THIS JOURNAL, **75**, 3287 (1953).