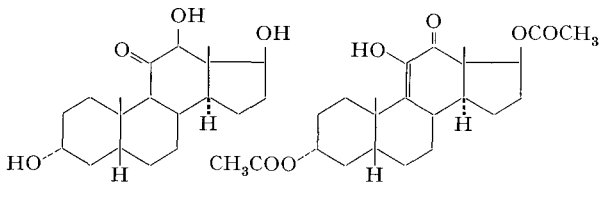
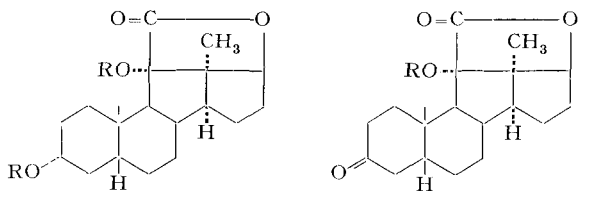
The Benzilic Acid Rearrangement of 3α,17β-Diacetoxy-11-hydroxy-12-oxo-5β-androst-9(11)-ene

The known[1] 3α,17β-diacetoxy-12-oxo-5β-androstane (*1*) was converted to a mixture of 11α- and 11β-bromoketones (*2*) following the procedure of JULIAN and MAGNANI[1]. Without separation of the isomers, this material was treated with sodium hydroxide in aqueous methanol and the new 11-oxo-3α,12β,17β-trihydroxy-5β-androstane (*3*)[2] was isolated and recrystallized from acetone; m.p. 235–236°; $[\alpha]_D^{27}$ + 52° (c, 0.546)[3]. The triolone (*3*) was oxidized with bismuth trioxide in acetic acid solution[4] and the 3α,17β-diacetoxy-11-hydroxy-12-oxo-5β-androst-9(11)-ene (*4*) was recrystallized from acetone; m.p. 247–248°; $[\alpha]_D^{26}$ + 108° (c, 1.106); λ_{max} 282 nm (ε 9600); ν_{max} 3400, 1725, 1667, 1600 cm^{-1}. The NMR-spectrum of *4* exhibited the expected C-10 methyl proton absorption at 72 c/sec[5].

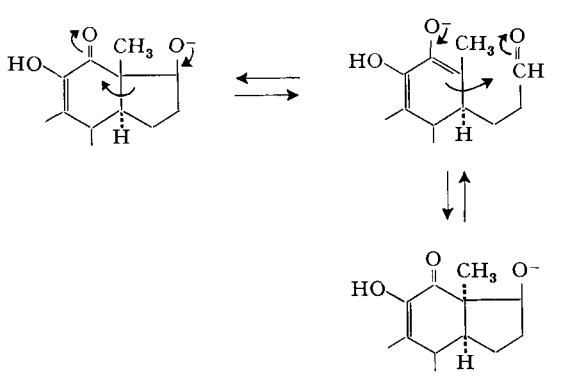


3 *4*



*5*a, R = H *6*a, R = H
*5*b, R = CH_3CO *6*b, R = CH_3CO

The intermediate *4* was treated with potassium hydroxide in aqueous propanol[6] in an atmosphere of nitrogen for 18 h under a gentle reflux to yield a new compound analyzing for $C_{19}H_{28}O_4$. An analytical sample was recrystallized from acetone-heptane; m.p. 187–188°; $[\alpha]_D^{26}$ − 76° (c, 1.011); no UV-absorption at 282 nm; ν_{max} 3600, 1755 cm^{-1}. The mass spectrum revealed the peak for the molecular ion at m/e 320 and 2 prominent peaks characteristic of the loss of 2 molecules of water at *M*-18 and *M*-36 which indicated that the compound was a diol. Oxidation with JONES reagent[7] converted only one of the two hydroxyl groups to a ketone. Thusit appearedt hat one of the hydroxyl groups was tertiary and it seemed possible that a benzilic acid rearrangement of *4* had occurred.



For stereochemical reasons it is impossible for a benzilic acid rearrangement product of *4* with the C/D *trans*-fused rings to form an 11a,17-lactone. An additional retroaldol equilibrium, preceding the benzilic acid rearrangement, which leads to epimerization at C-13 is postulated according to the equilibrium formulated below. This is required for γ-lactone formation of the benzilic acid rearrangement product in an all *cis*-fused system containing three 5-membered rings[8].

The product resulting from the alkali treatment of *4* was formulated as 11β-carboxy-3α,11α,17β-trihydroxy-13α-C-nor-5β-androstane 11a,17-lactone (*5*a) and the oxidation product was the ketone *6*a. The latter (*6*a) was recrystallized from acetone-heptane and the following physical constants were recorded: m.p. 247–249°; $[\alpha]_D^{26}$ − 59° (c, 1.014); ν_{max} 3578, 1755, 1700 cm^{-1}. In the mass spectrum, the molecular peak was observed at m/e 318 and a peak at *M*-18 indicated the presence of a hydroxyl group. Most important, a peak at *M*-70 (18%), characteristic of the loss of the first 4 carbon atoms in a 3-keto-5β-H steroid under electron impact[9], demonstrated that the A/B-ring system was not involved in the reaction that led to the destruction of the ring C chromophore during the alkali treatment of *4*. It was of particular interest to observe that the *M*-70 peak was missing in the parent alcohol *5*a; thus this peak is characteristic of the ketone *6*a. The conversion of *5*a to *6*a was accompanied by the disappearance of the broad NMR-absorption centered at about 218 c/sec for the 3β-axial proton while the multiplet centered at 258 c/sec attributed to the 17α-proton of *5*a was found unchanged in the product *6*a.

The rearrangement product *5*a gave rise to a diacetate *5*b, which was recrystallized from acetone-heptane to a constant melting point of 179–180°; $[\alpha]_D^{26}$ − 82° (c, 1.044); ν_{max} 1770, 1730 cm^{-1}. The hypsochromic shift of the lactone carbonyl absorption in the IR-spectrum of the acetylated product *5*b as compared to that of *5*a is consistent with the formulation of *5*a as an α-hydroxycar-

[1] P. L. JULIAN and A. MAGNANI, U.S. Pat. No. 2,940,991 (June 14, 1960).

[2] The triacetate of *3* was reduced with calcium in liquid ammonia by the method of J. H. CHAPMAN, J. ELKS, G. H. PHILLIPPS, and L. J. WYMAN, J. chem. Soc. *1956*, 4344, and the resulting product was acetylated to yield 3α,17β-diacetoxy-11-oxo-5β-androstane [L. H. SARETT, J. Am. chem. Soc. *69*, 2899 (1947)]. The latter compound was identified by its melting point, optical rotation and comparison of its IR-spectrum with that of authentic material.

[3] All compounds had satisfactory analyses for carbon and hydrogen. The optical rotations and the IR-spectra were recorded in chloroform solutions except where indicated otherwise. The UV-spectra were determined in methanol solutions. The NMR-spectra were determined with a Varian A-60 spectrometer with solutions in deuteriochloroform using tetramethylsilane as an internal reference. The mass spectra were obtained on a MAT mass spectrometer model CH 4.

[4] C. DJERSASSI, H. J. RINGOLD, and G. ROSENKRANZ, J. Am. chem. Soc. *76*, 5533 (1954); E. J. BECKER and A. COHEN, U.S. Pat. No. 3,185,713 (May 25, 1965).

[5] R. F. ZÜRCHER, Helv. chim. Acta *46*, 2054 (1963).

[6] H. R. NACE and D. H. NELANDER, J. org. Chem. *29*, 1677 (1964).

[7] K. BOWDEN, I. M. HEILBRON, E. R. H. JONES, and B. C. L. WEEDON, J. chem. Soc. *1946*, 39; C. DJERASSI, R. R. ENGLE, and A. BOWERS, J. org. Chem. *21*, 1547 (1956).

[8] R. ADAMS and N. J. LEONARD, J. Am. chem. Soc. *66*, 257 (1944), reported the all *cis*-fused anhydroplatynecine; R. B. WOODWARD, T. FUKUNAGA, and R. C. KELLY, J. Am. chem. Soc. *86*, 3162 (1964), formulated triquinacene as an all *cis*-fused system.

[9] H. BUDZIKIEWICZ and C. DJERASSI, J. Am. chem. Soc. *84*, 1430 (1962).

bonyl compound[10]. Further evidence for the proximity of the non-oxidizable hydroxyl group to the lactone carbonyl was obtained when the IR-spectra of the dihydroxy compound *5*a and the hydroxyketone *6*a were recorded in very dilute carbon tetrachloride solutions. The spectrum of the ketone *6*a showed only the absorption of the intramolecularly bonded hydroxyl (ν_{max} 3573 cm^{-1}), while the spectrum of the alcohol *5*a revealed absorptions for both the free and the intramolecularly bonded hydroxyl (ν_{max} 3620, 3575 cm^{-1})[11].

Finally, it was found that the oxidation product *6*a could be converted to the corresponding acetate *6*b, which exhibited the following physical constants: m.p. 176–178°; $[\alpha]_D^{26}$ − 97° (c, 0.896); ν_{max} 1770, 1750 shoulder, 1738 and 1700 cm^{-1}. In the mass spectrum of *6*b the absorption of the molecular ion at m/e 360 as well as the prominent peaks at *M*-60 and *M*-(60 + 70) were observed[12,13].

Zusammenfassung. Die Bildung von 11β-Carboxy-3α, 11α, 17β-trihydroxy-13α-C-nor-5β-androstan 11a,17-Lakton aus 3α, 17β-Diacetoxy-11-hydroxy-12-oxo-5β-$\Delta^{9(11)}$-androsten wird beschrieben. Der Mechanismus und die Stereochemie dieser Umwandlung (Retro-Aldolkondensation und Benzilsäureumlagerung) wird diskutiert.

P. KURATH

Organic Chemistry Department, Research Division, Abbott Laboratories, North Chicago (Illinois 60064, USA),
June 24, 1966.

[10] W. NEUDERT and H. RÖPKE, *Steroid-Spektrenatlas* (Springer-Verlag Inc., New York 1965), Spectra Nos. 389 and 390.
[11] Cf. M. TICHÝ, in: *Advances in Organic Chemistry: Methods and Results* (Interscience Publishers, New York 1965), vol. 5, pp. 119, 162, and 210.
[12] The author is indebted to Mrs. BRIGITTE FRUEHWIRTH for IR-spectra, to Mrs. RUTH STANASZEK for NMR-spectra, and to Mr. O. L. KOLSTO for microanalyses.
[13] I wish to express my thanks to Dr. W. COLE and Dr. J. TADANIER of Abbott Laboratories, and to Dr. P. BEAK of the University of Illinois for stimulating discussions.

The Effect of Nicotinamide Adenine Dinucleotide on Lipolysis in Adipose Tissue in vitro

Lipolysis in adipose tissue in vitro has been shown to be stimulated by a variety of sympathomimetic amines and peptide hormones[1,2]. There is evidence that these effects are mediated by the activation of adenyl cyclase, production of cyclic-3′,5′-AMP[3], activation of an adipose tissue lipase[4,5], hydrolytic cleavage of adipose tissue triglycerides and subsequent release of fatty acids and glycerol. Inhibition of lipolysis induced by the sympathomimetic amines or peptide hormones has been demonstrated with a number of compounds, including nicotinic acid[6] and various metabolites of nucleic acids[7]. Nicotinic acid has been demonstrated to be a potent lipolysis inhibitor in vitro and this effect can be observed in vivo in the form of a pronounced depression of plasma non-esterified fatty acids (NEFA)[8]. DOLE[7] has reported that ATP, 5′-AMP and adenosine inhibited lipolysis, whereas the purines, adenine and guanine, and the pyrimidines, uracil and cytosine, increased lipolysis. More recently, MATSUZAKI and RABEN[9], differing from DOLE, found that guanine inhibited the lipolytic action of epinephrine. They also reported that adenine stimulated lipolysis at high concentrations but had no significant effect at lower levels.

Nicotinic acid has been shown to be an effective precursor for nicotinamide adenine dinucleotide (NAD) synthesis[10]. The possibility exists that adenosine might also contribute to NAD synthesis. In view of the fact that both nicotinic acid and adenosine have been shown to inhibit lipolysis in vitro, it was of interest to investigate the antilipolytic effects of NAD and related compounds.

In order to define the lipolysis inhibitory effects of NAD and related compounds, epididymal adipose tissue was taken from male Sprague-Dawley rats, 180–240 g, fed ad libitum. The tissue was placed in freshly aerated Krebs-Ringer bicarbonate buffer, pH 7.4, and minced with scissors into pieces weighing approximately 10 mg. 200 ± 3 (mean ± standard deviation) mg of tissue were placed in each experimental flask containing 3 ml of freshly aerated (95% O_2-5% CO_2) Krebs-Ringer bicarbonate buffer, pH 7.4. Bovine plasma albumin, fraction IV, 1%, was used as a fatty acid acceptor in this incubation medium. Sufficient norepinephrine (20–30 ng/ml) to cause 50% of maximum fatty acid release was added to the incubation mixture. The compounds under investigation were then added at appropriate concentrations. The experimental flasks were stoppered, aerated with 95% O_2-5% CO_2 for 10 min and incubated at 37 °C for 3 h on a Dubnoff metabolic shaker. After incubation, aliquots were removed and analyzed for fatty acid content by the method of DOLE[11]. The fatty acids released into the medium by norepinephrine were compared with the release of fatty acids in flasks containing norepinephrine and the test compound. The effect of the inhibitor was expressed in terms of % inhibition.

Under the conditions of the study, NAD was shown to be a potent inhibitor of the norepinephrine-induced fatty acid release from adipose tissue (Table I). This inhibition was proportional to concentration over a range of 10^{-4} to 10^{-7} *M*. NAD is composed of nicotinamide mononucleotide (NMN) and 5′-AMP joined through a pyrophosphate bridge. NMN itself was a comparatively weak inhibitor of fatty acid release as was nicotinamide. Nicotinic acid, on the other hand, was a very potent inhibitor. The

[1] J. E. WHITE and F. L. ENGEL, Proc. Soc. exp. Biol. Med. *99*, 375 (1958).
[2] M. VAUGHAN and D. STEINBERG, J. Lipid Res. *4*, 193 (1963).
[3] R. W. BUTCHER, R. J. Ho, H. C. MENG, and E. W. SUTHERLAND, Abstr. Sixth Int. Union Biochem. *32*, 715 (1964).
[4] M. A. RIZACK, J. biol. Chem. *239*, 392 (1964).
[5] M. VAUGHAN, J. E. BERGER, and D. STEINBERG, J. biol. Chem. *239*, 401 (1964).
[6] L. A. CARLSON, Acta med. scand. *173*, 719 (1963).
[7] V. P. DOLE, J. biol. Chem. *236*, 3125 (1961).
[8] L. A. CARLSON and L. ORÖ, Acta med. scand. *172*, 641 (1962).
[9] F. MATSUZAKI and M. S. RABEN, Fedn Proc. Am. Socs exp. Biol. *24* (2), 342 (1965).
[10] H. B. BURCH, C. A. STORVICK, R. L. BICKNELL, H. C. KUNG, L. G. ALEJO, W. A. EVERHART, O. H. LOWRY, C. G. KING, and O. A. BESSEY, J. biol. Chem. *212*, 897 (1955).
[11] V. P. DOLE, J. clin. Invest. *35*, 150 (1956).