series tertiary > secondary > primary. Since the rate of aliphatic nitro radical anion decomposition seems to reflect the relative stability of radical anions, we shall use this parameter to study such species.

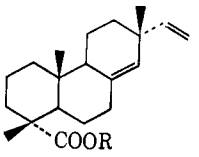
Acknowledgment. We wish to acknowledge the support of the following groups: Research Corporation, North Carolina Board of Science and Technology, and the University of North Carolina at Charlotte Foundation. We express our appreciation to the Computer Center of the University of North Carolina at Charlotte for their assistance.

R. H. Gibson, J. C. Crosthwaite
Department of Chemistry
University of North Carolina at Charlotte
Charlotte, North Carolina 28205
Received September 9, 1968

The Synthesis of (−)-Sandaracopimaric Acid

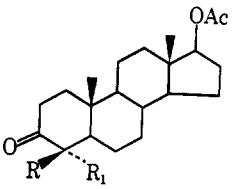
Sir:

We wish to report the synthesis of (−)-sandaracopimaric acid (Ia) from testosterone. This synthesis provides a direct confirmation of the assigned structure and stereochemistry for the natural acid.[1]

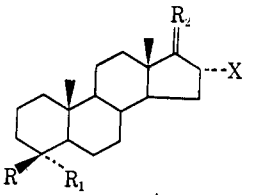


Ia, R = H
b, R = CH_3

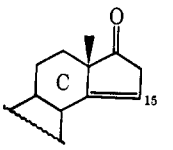
Reductive carbomethoxylation of testosterone acetate using the Stork procedure[2,3] afforded IIa in 24% yield, mp 159–161°, $[\alpha]D$ −2.8°, nmr, δ 3.28 (1 H, doublet, J = 12 cps), 3.76 (3 H, singlet).[4] Methylation[5] of IIa proceeded stereoselectively to afford IIb in 64% yield, mp 166–168°, $[\alpha]D$ −24.1°, and some IIc, mp 200–201°, $[\alpha]D$ −7.3°; the ratio of IIb to IIc formed in this alkylation is 9.2:0.8. Clemmensen reduction of the keto esters IIb and IIc afforded the corresponding 3-deoxy esters[6] IIIa, mp 173–178°, $[\alpha]D$ −6.7°, and IIIb, mp 180–184°, $[\alpha]D$ +37.1°.



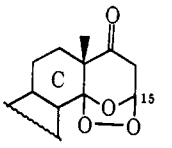
IIa, R = H; R_1 = CO_2CH_3
b, R = CH_3; R_1 = CO_2CH_3
c, R = CO_2CH_3; R_1 = CH_3



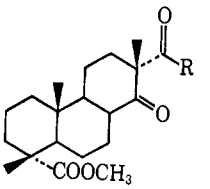
IIIa, R = CH_3; R_1 = CO_2CH_3
b, R = CO_2CH_3; R_1 = CH_3
} R_2 =  , X = H
c, X = H
d, X = Br
} R = CH_3; R_1 = CO_2CH_3; R_2 = O



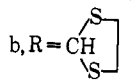
IV



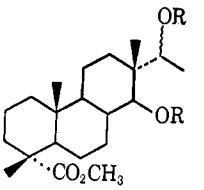
V



VIa, R = CH=CH—OH



b, R = CH
c, R = CH_3



VIIa, R = H
b, R = C_6H_5CO

Oxidation of IIIa with Jones reagent afforded IIIc, mp 140–142°, $[\alpha]D$ +42.9°. Enol acetylation of IIIc followed by bromination[7] afforded the 16α-bromo ketone IIId, mp 220–224°, $[\alpha]D$ +51.9°, which on dehydrobromination with lithium bromide–lithium carbonate in DMF[8] gave the deconjugated ketone IV as the major product, mp 102–103°, $[\alpha]D$ +104.6°. Treatment of IV with ozone at −70° led to the formation of a stable ozonide V, mp 179–185°, in quantitative yield. The protons at C_{15},C_{16} exhibit an ABX splitting pattern in the nmr, δ(H_A) 2.58, δ(H_B) 2.86 (2 H, octet, J_{AB} = 17.5, J_{AX} = 1.5, and J_{BX} = 3.0 cps), δ(H_X) 5.96 (1 H, multiplet, $J_{AX + BX}$ = 5.0 cps). Cleavage of the ozonide by catalytic hydrogenation in the presence of palladized carbon gave the hydroxymethylene ketone VIa, λ_{max}^{MeOH} 259 mμ (ε 3000) → $\lambda_{max}^{MeOH-OH^-}$ 296 mμ (ε 13,000). Decarbonylation[9] by interacting VIa with ethylene *p*-toluenethiolsulfonate[10] proceeded smoothly to afford the thioketal VIb, mp 158–161°, $[\alpha]D$ −14.1°, nmr, δ 5.00 (1 H, singlet), 3.35

(1) (a) O. E. Edwards, A. Nicholson, and M. N. Rodger, *Can. J. Chem.*, **38**, 663 (1960); (b) V. Galik, J. Kulhan, and F. Petru, *Chem. Ind.* (London), 722 (1960); (c) A. K. Bose, *ibid.*, 1104 (1960); (d) R. E. Ireland and P. W. Schiess, *J. Org. Chem.*, **28**, 6 (1963).

(2) G. Stork, P. Rosen, N. Goldman, R. V. Coombs, and J. Tsuji, *J. Am. Chem. Soc.*, **87**, 275 (1965).

(3) T. Spencer, T. D. Weaver, R. M. Villarica, R. J. Friary, J. Posler, and M. A. Schwartz, *J. Org. Chem.*, **33**, 712 (1968).

(4) Satisfactory elemental analyses and spectral data were obtained for all new compounds. Specific rotations were determined on 0.3% solutions in dioxane. Nmr spectra in $CDCl_3$ using TMS as the internal standard were determined on a Varian A-60A spectrometer. The author thanks Mr. M. Yudis and his staff for these physical measurements.

(5) The alkylations were conducted by generating the anion of the keto ester with sodium hydride in refluxing benzene followed by treatment with methyl iodide. A study of the stereochemistry of alkylation of some steroidal keto esters will be included in the full paper.

(6) The stereochemical assignments at C_4 in these compounds were made on the basis of the following evidence. A 3-hr basic hydrolysis at 150° of the equatorial ester IIIa gave a quantitative yield of the corresponding acid, whereas the axial ester IIIb was stable under these conditions. The 19-methyl resonance of IIIb appears at δ 0.70, shielded by 0.19 ppm relative to the corresponding resonance of IIIa at δ 0.89. This 1,3-diaxial shielding effect by a carbonyl group is reported in the tricyclic [E. Wenkert, A. Afonso, P. Beak, R. W. J. Carney, P. W. Jeffs, and J. D. Chesney, *J. Org. Chem.*, 30, 713 (1965)] and bicyclic (*cf.* ref 3) series.

(7) R. Pappo, B. M. Bloom, and W. S. Johnson, *J. Am. Chem. Soc.*, **78**, 6347 (1956).

(8) R. Joly, J. Warnant, G. Nominé, and D. Bertin, *Bull. Soc. Chim. France*, 366 (1958).

(9) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *J. Chem. Soc.*, 1131 (1957).

(10) The author thanks Dr. I. Pachter of Endo Laboratories for a generous gift of this reagent.

(4 H, multiplet). Desulfurization of VIb with W-2 Raney nickel gave a quantitative yield of the methyl ketone VIc, mp 122–123°, nmr, δ 2.14 (3 H, singlet). Catalytic reduction of the diketone in the presence of platinum afforded the diol VIIa as a two-component mixture of epimers at C_{15}. The final steps parallel those used in the synthesis of (−)-sandaracopimaradiene.[11] Thus, the diols VIIa without separation were benzoylated and the dibenzoate mixture VIIb was pyrolyzed at 440°. There was obtained a 55% yield of methyl sandaracopimarate (Ib). Ester cleavage of Ib with lithium iodide in collidine[12] gave (−)-sandaracopimaric acid (Ia), mp 165–168°, undepressed on admixture with authentic[13] Ia, $[\alpha]_D$ −19.8° (*c* 0.2, ethanol) [lit.[1a] $[\alpha]_D$ −20°]. The infrared spectra and mobilities on thin-layer chromatography of the natural and synthetic Ia were identical.

(11) P. Johnston, R. C. Sheppard, C. E. Stehr, and S. Turner, *J. Chem. Soc., C*, 1847 (1966).
(12) F. Elsinger, J. Schreiber, and A. Eschenmoser, *Helv. Chim. Acta*, **43**, 113 (1960).
(13) The author thanks Dr. O. E. Edwards for a sample of natural sandaracopimaric acid.

Adriano Afonso
Natural Products Department, Schering Corporation
Bloomfield, New Jersey 07003
Received September 23, 1968

Relative Reactivities of *p*-Nitrophenyl Phosphate and Phosphorothioate toward Alkaline Phosphatase and in Aqueous Hydrolysis

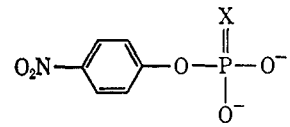
Sir:

There have been several reports of enzymatic studies on sulfur analogs of normal phosphate ester substrates. Thus Neumann, *et al.*,[1] find that S esters of phosphorothioic acid are rapidly cleaved by *E. coli* alkaline phosphatase. However, Eckstein has reported that nucleoside 5′-phosphorothioates are inert to both alkaline phosphatases[2] and some,[3] but not all,[4] acid phosphonoesterases; in contrast to these P=S compounds, the P=O derivatives are of course rapidly hydrolyzed. Recently[4] Neumann has reported that *p*-nitrophenyl phosphorothioate (I) is inert to *E. coli* alkaline phosphatase.

We have also investigated the hydrolysis of I by *E. coli* alkaline phosphatase[5] and find that while I is hydrolyzed slowly relative to the normal phosphate substrate II, I is by no means enzymatically inert. In fact the rate ratio for the sulfur derivative II and oxygen analog I can be explained chiefly on a straightforward chemical basis and furnishes a new type of evidence on the enzyme mechanism.

(1) H. Neumann, L. Boross, and E. Katchalski, *J. Biol. Chem.*, **242**, 3142 (1967).
(2) F. Eckstein, *J. Am. Chem. Soc.*, **88**, 4292 (1966).
(3) F. Eckstein and H. Sternbach, *Biochim. Biophys. Acta*, **146**, 618 (1967); *cf.* also H. Matzura and F. Eckstein, *European J. Biochem.*, **3**, 448 (1968).
(4) H. Neumann, *J. Biol. Chem.*, **243**, 4671 (1968).
(5) Chromatographically purified *E. coli* alkaline phosphatase, Worthington Biochemical BAPC7 JB, was further purified by a procedure developed by Professor Wilmer Fife. The enzyme was centrifuged at 3600 rpm with 65% saturated ammonium sulfate solution at 0° for 30 min, 10 mg of the solid was dissolved in 1 ml of 0.1 *M* N-ethylmorpholine buffer pH 8.0–0.1 *M* NaCl, and the residue was removed at 5000 rpm, 0°, 30 min. This stock solution was diluted tenfold in buffer to produce the working solution, *ca.* 1 mg/ml. Enzyme concentration in each reaction is approximately 3×10^{-2} mg/ml.

Solutions of I were prepared by dissolving *p*-nitrophenylthiophoshoryl dichloridate[6] in dioxane and hydrolyzing in a pH-stat at pH 8.0 with 1 *N* aqueous NaOH. Base uptake ceased after 4.0 equiv and spectroscopic assay indicated *ca.* 2% *p*-nitrophenol. Kinetic studies (*vide infra*) indicated minor contamination by II. I was stable in solution, but decomposed on attempted isolation under a variety of conditions, so the solution of I was utilized in hydrolysis studies.



I, X = S
II, X = O

The hydrolysis of I in 0.1 *N* N-ethylmorpholine buffer–1 *N* NaCl, pH 8.0, 25°, was followed with a Cary spectrophotometer at 410 mμ.

Contamination of I by a small amount of II led to a rapid initial rate and interfered with the normal K_m determination; however, hydrolysis of I is competitively inhibited by inorganic phosphate, so K_m for I was determined to be 1.3×10^{-4} *M* from the observed rate effect of added phosphate and its known inhibition constant. V_{max} was determined in the usual fashion to be 5.9×10^{-8} *M* sec^{-1}; V_{max} for II under the same conditions is 6.3×10^{-6} *M* sec^{-1} and $K_{mII} = 1.5 \times 10^{-5}$ *M*. Thus K_m for the thiophosphate ester is *ca.* eight times greater than for the oxygen ester, as has been observed in another case.[3] The major difference between I and II is that k_{catII} is $100k_{catI}$.

By contrast, in nonenzymatic aqueous solution I hydrolyzes much more rapidly than II. Both hydrolyses[7] are first order in H^+, and the two compounds have different pK_a's, so near the pK_a the rate ratio is pH dependent. At pH 8.0, 70°, k_I/k_{II} is 48, while at pH 7.0, 70°, $k_I/k_{II} = 63$. Ketelaar[8] has reported relative rates of alkaline hydrolysis for a series of phosphate and thiophosphate triesters and found a rate ratio k_S/k_O of *ca.* 0.03. The inversion in the S/O rate ratio in phosphate monoester *vs.* triester aqueous hydrolyses is reasonable. It is well established that triesters follow an addition–elimination mechanism, in which the P=O (P=S) bond order decreases in the transition state and the oxygen (sulfur) increases in charge, while the monoesters use an elimination (to metaphosphate)–addition sequence, in which the P=O (P=S) bond order increases in the transition state and the charge on oxygen (sulfur) diminishes. The effects themselves probably reflect mainly the lesser electronegativity of sulfur compared with oxygen.

The S/O rate ratio for the enzymatic hydrolysis of I and II resembles that for alkaline hydrolysis of triesters and is the inverse of that for nonenzymatic hydrolysis of I and II. This points strongly to an addition–elimination sequence for alkaline phosphatase. While there is some difference in binding of I and II as well, it seems unlikely that this could so drastically invert the

(6) H. Tolkmith, *J. Org. Chem.*, **23**, 1685 (1858).
(7) We confirm the data of K. Holbrook and L. Ouellet, *Can. J. Chem.*, **36**, 686 (1958), on the hydrolysis of II.
(8) J. Ketelaar, H. Gersmann, and K. Koopmans, *Rec. Trav. Chim.*, **71**, 1253 (1952).