nan-3β,11β,17α,21-tetraol-20-one (Reichstein's substance V).[9]

Cyclization of the *dl*-diesters with potassium *t*-butoxide, followed by hydrolysis and decarboxylation, accomplished by heating with aqueous dioxane at 200–210°, gave after saponification *dl*-3β,11β-dihydroxyandrostane-17-one (VII), m.p. 249–251.5° (C, 74.4; H, 9.61), and the 13-iso compound, m.p. 216–217° (C, 74.3; H, 9.73). The diacetate of the former isomer melted at 217–217.5° (C, 70.7; H, 8.97) and had a characteristic infrared spectrum which was identical with that of authentic *d*-VII diacetate, m.p. 153.5–155°.[10,11]

It is noteworthy that when the acidic product from the ozonization in the 13-iso series was saponified to remove the acetate residues, and then isolated in the usual manner by acidification, the product was a lactonic acid, m.p. 264–266° (C, 68.4; H, 8.83; neut. equiv., 358), λ_{max}^{Nujol} 2.92 μ, 5.73 μ, 5.88 μ. The 5.73 μ band is indicative of a γ-lactone, and the compound is therefore represented by formula V. Since there is no doubt about the configuration at C_{13} in this compound, the formation of this lactone establishes unequivocally that the hydroxyl group at C_{11} is in the β-configuration. This observation coupled with the relationship of the C_{13} epimeric series to the natural steroids, thus constitutes conclusive confirmation of the C_{11} configuration of the natural 11-hydroxy steroids.

We are deeply grateful to the Research Committee of the Graduate School of the University of Wisconsin and to the Sterling–Winthrop Research Institute for generously supporting this program. We are also indebted to these agencies as well as to Merck and Co., Inc., for aiding the work in the 11-desoxy series.[3]

(9) We are indebted to Dr. G. Rosenkranz of Syntex, S. A., for supplying us with this substance and for providing us with unpublished details for its oxidation with sodium bismuthate.

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(11) We wish to thank Dr. Max Tishler of Merck and Co., Inc., for supplying us with 17α-hydroxycorticosterone from which this product was prepared.

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1,2,3,4-TETRAPHENYLFULVALENE

Sir:

Recent theoretical calculations[1,2,3,4] predicting the properties of the hitherto non-existent fulvalene hydrocarbons has stimulated interest in their synthesis. It is the purpose of this Communication to report the synthesis of the first non-fused ring fulvalene, 1,2,3,4-tetraphenylfulvalene (I).

The addition of cyclopentadienylmagnesium bromide[5] to tetracyclone in a mixture of ethyl ether and benzene afforded 74% of the almost colorless 1-cyclopentadienyltetraphenylcyclopentadiene-1-ol (II), m.p. 197.6–198.6°. Calcd. for $C_{34}H_{26}O$: C, 90.63; H, 5.82. Found: C, 90.43; H, 6.00 (λ_{max} 242 mμ, log ϵ 4.45; λ_{max} 337 mμ, log ϵ 3.85 in methanol). The infrared showed a band at 2.83 microns, but none in the region 5.6–6.1 microns indicating the presence of an hydroxyl group, no carbonyl, and thus that 1,2-addition, and not 1,4-addition, had taken place.

In refluxing bromobenzene II gave a colorless maleic anhydride adduct (III), m.p. 251–252.5° (dec.). Calcd. for $C_{38}H_{28}O_4$: C, 83.2; H, 5.1. Found: C, 82.9; H, 5.4 (λ_{max} 223 mμ, log ϵ 4.43; λ_{max} 265 mμ, log ϵ 4.08). Strong bands at 5.42 and 5.65 microns are characteristic of the anhydride ring, band at 2.90 microns indicates the hydroxyl group.

Dehydration of II was effected with iodine in boiling benzene to give the brilliant orange-red I in 27% yield, m.p. 201–202°. Calcd. for $C_{34}H_{24}$: C, 94.41; H, 5.59. Found: C, 94.04; H, 5.51 (λ_{max} 278 mμ, log ϵ 4.34; $\lambda_{infl.}$ 320, log ϵ 3.07; λ_{max} 415 mμ, log ϵ 2.95). No hydroxyl band is present in the infrared.

When III was heated at 275° for 10 minutes 98% of the theoretical quantity of water is eliminated. The red product (IV), obtained in 20% yield, melts at 177–181°. Calcd. for $C_{38}H_{26}O_3$: C, 86.01, H, 4.94. Found: C, 85.93; H, 4.6 (λ_{max} 240 mμ, log ϵ 4.37; $\lambda_{infl.}$ 315 mμ, log ϵ 3.91; $\lambda_{infl.}$ 415 mμ, log ϵ 2.93). There was no band at 2.90 microns; the bands at 5.42 and 5.65 microns were still present. This anhydride did not depress the melting point of the maleic anhydride adduct from I in boiling toluene in 40% yield, m.p. 178–179° (m.m.p. 178–181°). The red color of the maleic anhydride adduct indicates that the phenylated fulvene system is intact in the molecule since non-phenylated fulvenes are yellow. Thus, maleic anhydride must add to the unsubstituted C-5 ring in both I and II.

The authors express their appreciation to Charles Pfizer Co., Inc., in whose laboratories this work was done, for their encouragement.

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THIOESTERASES FOR ACYL AND AMINOACYL MERCAPTANS[1]

Sir:

The recognition of the function of acyl mercaptans in biosynthetic processes[2] suggests a possible role of substituted or unsubstituted α-aminoacyl mercaptans as intermediates in peptide synthesis.[3,4,5] Such a concept would be supported by the occurrence of hydrolyzing and/or transferring thioesterases with a specificity directed toward the amino acid portion of the thioester. We wish to report on the presence in ox brain cortex and liver of a group of thioesterases able to catalyze the hydroly-

(1) This work was supported in part by grants from the National Institute of Neurological Disease and Blindness (Grant B-226) of the National Institutes of Health, Public Health Service, and by a contract between the Office of Naval Research and the Psychiatric Institute.

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sis of various aliphatic acyl and aminoacyl mercaptans of glutathione (GSH) and N-acetylcysteine.

Of the following acyl mercaptans used as substrates S-acetyl-, S-crotonyl-, S-L-alanyl-, S-acetylglycyl-GSH and S-N-diacetylcysteine were prepared by modifications of known procedures.[6-10] S-L-α- and S-L-γ-glutamyl-GSH were prepared by reacting α- or γ-carbobenzyloxy-thioglutamic acid with GSH and subsequent removal of the carbobenzyloxy moiety with hydrogen bromide in phenol-acetic acid.

The acyl mercaptans were determined as hydroxamic acids after reaction with hydroxylamine. The data in the table record the specific activities, with the various acyl mercaptans as substrates, of several ammonium sulfate fractions of a liver extract. The ratio of specific activity with any substrate except S-L-α-glutamyl-GSH to the specific activity with S-acetyl-GSH as the substrate changed from one fraction to another. The table lists those fractions in which the highest and lowest ratios were found. The ratios of rates of hydrolysis of S-L-α-glutamyl- and S-acetyl-GSH, respectively, varied with the different enzyme fractions over a narrower range than those of any of the other substrates. The present data do not exclude the possibility that α-glutamyl- and acetyl-GSH are hydrolyzed by the same enzyme while the hydrolysis of the other acyl mercaptans appears to be catalyzed by enzymes distinct from each other as well as from the S-acetyl-GSH thioesterase. Because of its instability comparative data on the enzymatic hydrolysis of S-L-γ-glutamyl-GSH could not be obtained. The specificity of the thioesterases was not only directed towards the acyl moiety but also towards the mercaptan as demonstrated by the different rates of hydrolysis for S-acetyl-GSH and S-N-diacetylcysteine in different fractions.

TABLE I

THIOESTERASE ACTIVITY OF FRACTIONS OF LIVER (OX)

S.A.$_1$ specific activity of ammonium sulfate fraction with substrate listed; S.A.$_2$ specific activity with S-Acetyl-GSH as substrate. All values corrected for non-enzymatic splitting of thioesters.

	S.A.$_1$	S.A.$_2$	S.A.$_1$/S.A.$_2$	Fractions
S,N-Diacetyl-cys-	0	15.7	0	C
teine	7.3	11.1	0.66	A
S-Acetylglycyl-	16.7	11.1	1.5	A
GSH	79.0	15.7	4.9	C
S-Crotonyl-GSH	8.0	21.5	0.37	Orig.
	14.8	15.7	0.94	C
S-L-Alanyl-GSH	12.3	11.2	1.1	B
	39.3	15.7	2.5	C
S-α-Glutamyl-GSH			1.4–1.6	(9 fractions)

Of S-alanyl-GSH 5 μM. and of the other substrates 2.5 μM. were incubated with 0.15 to 0.5 mg. of protein and 50 μM. tris-hydroxymethyl-aminomethane (*p*H 7.6) in a total volume of 1 ml. of 5 μM. S-L-α-glutamyl-GSH were incubated with 50 μM. acetate (*p*H 5.5) and the same amounts of protein. Incubation 30′ at 30°. Orig. = extract of acetone-dried powder with 0.02 *M* K_2HPO_4 (*p*H 7.4). Fraction A was obtained between 28–36% $(NH_4)_2SO_4$ saturation of original extract. Fractions B and C were obtained by refractionation of the combined 0–28 and 36–64% fractions at 0–28, and 36–48% $(NH_4)_2SO_4$ saturation, respectively.

Our observations do not indicate whether the thioesterases communicated from other laboratories[11,12,13] while this work was in progress, are different from some of those reported here.

It will be of particular interest to ascertain the activity of these enzymes in hydrolyzing amide and O-ester linkages and in catalyzing the transfer of the acyl and aminoacyl radicals to acceptors other than water.

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THE ROLE OF MOLYBDENUM AND FLAVIN IN HYDROGENASE

Sir:

Clostridium pasteurianum, an obligate anaerobic bacterium that fixes elemental nitrogen contains relatively large amounts of hydrogenase. Cell-free extracts of this enzyme were prepared by subjecting the organism to sonic vibrations and the enzyme was purified by serial application of the following procedures: separation of the particulate fraction by high speed centrifugation, removal of impurities first with protamine sulfate, followed by zinc hydroxide gel, and finally fractional precipitation with ammonium sulfate. The enzyme was not sedimented during a thirty-minute exposure to 144,000 $\times$ gravity.

The activity of the enzyme was followed manometrically—the assay being based on the oxidation of molecular hydrogen by methylene blue. At the highest purity level obtained, 1 mg. of protein N catalyzed the oxidation of 750,000 μl. of hydrogen per hour at 30°. Purified preparations were stable for two weeks when stored at 2°, under hydrogen, in presence of 30–40% ammonium sulfate and at *p*H 6–6.5. The enzyme was inactivated by exposure to oxygen.

While crude preparations of the enzyme readily catalyzed the oxidation of hydrogen by mammalian cytochrome *c*, this property was either reduced or abolished in the purified preparation. However, full activity could be restored by addition to the purified extracts of molybdenum in the form of MoO_3. Molybdenum could not be replaced by Mn^{++}, Mg^{++}, Fe^{++}, Fe^{+++}, Cu^{+}, Cu^{++}, Co^{++}, $WO_4^{=}$ or VO_3^{-}. The presence of inorganic phosphate was required for the catalysis of cytochrome *c* reduction by molybdenum. The dissociation of molybdenum from the enzyme takes place throughout the purification procedure. However, the presence of the metal in bound form is demonstrable at all levels of purity.

When the absorption spectrum of the enzyme ox-