with or without imidazole, in air or nitrogen atmosphere, at temperatures between −28° and +24°, and over periods of 18 hours to 18 days. Concentrated solutions of K 6-APA (>2 molal) form a precipitate on standing at room temperature for about 60 hours. This precipitate can be redissolved and is chemically similar to the product remaining in solution.

β-Lactam cleavage was revealed by loss of the 5.6–5.7 μ band in infrared spectra and loss of the capacity to react with hydroxylamine at neutral pH. Formation of monosubstituted amide linkages was revealed by appearance of 6.0 and 6.6 μ absorption bands and by *loss* of ninhydrin reactivity, after OH^- treatment, which paralleled loss of hydroxylamine reactivity. Differences between 6-APA, penicic acid, 8-hydroxypenillic acid (8-HPA)[2−4] and 6-APA peptide are shown in Table I. Peptide A was formed from 5 molal K 6-APA in water at 23° for 3 days, then 24-hour dialysis, and freeze-drying. Peptide B was formed from 3 molal K 6-APA in water, under nitrogen, at 23° for 18 days, and freeze-drying.

TABLE I

COMPARISON OF 6-APA PEPTIDES AND RELATED COMPOUNDS

	6-APA	8-HPA	Peptide A	Peptide B
Hydroxamate[a]	100.0	0.0	12.2	2.6
Ninhydrin—direct[b]	0.498	.004	0.142	0.162
Ninhydrin—after OH^-[b]	1.076	.020	0.148	0.204
6.0 and 6.6 μ bands	−	−	+	+

[a] % intact β lactam as 6-APA. [b] Net optical density in the assay for 0.1 mg. of product.

Paper electrophoresis of peptide B, 6-APA, and 8-HPA (pH 4.48, 13 volts/cm., 22°, 2.5 hours), and ninhydrin staining, showed that 6-APA did not migrate, 8-HPA did not stain, and B moved, as 3 non-discrete spots, distances of 1–3 cm. toward the anode. Peptide A, with 88% of β-lactam lost, had no antimicrobial activity against *Staph. aureus* before or after acylation with phenylacetyl chloride. $[\alpha]^{1\%}_D$ was +162.2° for A, compared with +286.9° for K 6-APA. The number average molecular weight for the acid product is 1770, calculated from the residual β-lactam groups, and 1570, calculated from the residual α-amino groups. These data indicate a seven or eight unit peptide.

The amount of product failing to pass through cellulose dialysis membranes varied with preparative conditions. Thus, peptide A represented 20% of the initial 6-APA. Subsequent 6-hour dialysis of A and of K 6-APA (8/32 Visking casing) showed retention of 65% of polymer and 6% of K 6-APA. Another peptide, C, formed from 0.1 *M* K 6-APA in the presence of 0.1 *M* imidazole at −18° in 18 hr., was retained to the extent of 70% after prolonged dialysis. C showed absorption maxima at 6.0 and 6.6 μ, but not at the β-lactam region near 5.7. Elemental analysis gave: C, 37.4; H, 5.1; N, 11.7. Calcd. for $C_8H_{11}O_3N_2SK$: C, 37.8; H, 4.3; N, 11.0. Light scattering indicated a molecular weight of about 2500 (range 1700–3400) for the acid peptide. The ninhydrin assay showed eight units in the peptide, giving a molecular weight of 1730.

In mixtures of 6-APA and penicillin G or V, the β-lactam of 6-APA is preferentially attacked by the nucleophilic amino group. This was observed in systems at −18° for 2 weeks or at 23° for 3 days. Paper chromatography showed no new antibiotic formed, and amyl acetate extracts of acidified solutions appeared to contain only the initial penicillin. This finding is surprising, inasmuch as penicillin G and V β-lactams are more susceptible than that of 6-APA to base and penicillinase attack.

Although 6-APA differs from the structural units of proteins, the fused β-lactam is an intriguing addition to the activated groups involved in peptide bond formation.

Acknowledgment.—The authors wish to thank Dr. W. Reiss for providing the physical and elemental analyses and Mr. R. Whitley for the hydroxamate assays.

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(2) D. A. Johnson and G. A. Hardcastle, Jr., *J. Am. Chem. Soc.*, **83**, 3534 (1961).

(3) A. Ballio, E. B. Chain, F. Dentice Di Accadia, M. Mauri, K. Rauer, M. J. Schlesinger and S. Schlesinger, *Nature*, **191**, 909 (1961).

(4) F. R. Batchelor, D. Gazzard and J. H. C. Nayler, *ibid.*, **191**, 910 (1961).

TRANSFORMATIONS OF EBURICOIC ACID. SIDE CHAIN DEGRADATION TO PREGNANE DERIVATIVES

Sir:

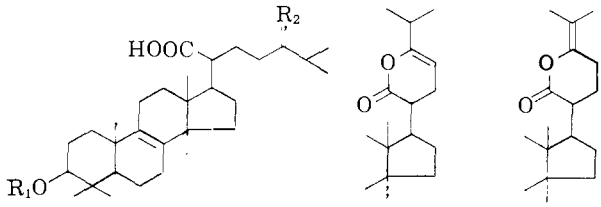
The overwhelming majority of the therapeutically important steroid hormones and hormone analogs currently in use are derived by partial synthesis from precursors of animal or plant origin. The dominant position occupied by these raw materials has been challenged, although not as yet effectively, by steroids derived by microbial biosynthesis, among which the yeast sterol ergosterol has been the one to receive almost undivided attention. The growing trend during recent years to introduce additional structural elements such as alkyl, halogen or unsaturation into steroid hormones to enhance or modify their activities has stimulated our interest in the triterpenoid methyl steroids, another group of microbiologically derived products, among which eburicoic acid (I)[1,2] appeared particularly attractive because of its abundance in the mycelium (25–30% of the dried weight) and its ready production under submerged culture conditions.[3] This paper describes the degradation of eburicoic acid to derivatives possessing the 2-carbon side chain of progesterone

(1) For a review of the chemistry of eburicoic acid see Sir J. Simonsen and H. C. J. Ross in "The Terpenes," Vol. 5, The University Press, Cambridge, England, 1957, p. 1 ff.

(2) After completion of this work E. Graf and H. Winckelman (*Arch. Pharm.*, **294**, 413 (1961)) reported on attempts to convert eburicoic acid into 11-keto corticosteroids.

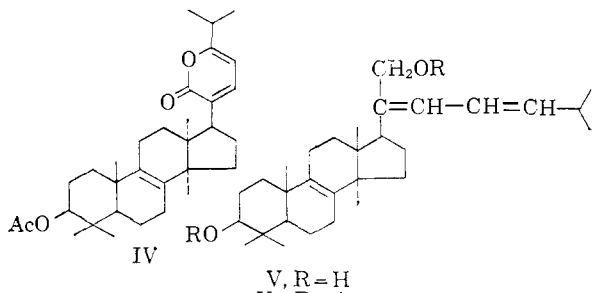
(3) S. C. Pan and L. J. Lerner, U. S. Patent 3,010,878.

and corticosterone without impairment of the sensitive 8,9-double bond.



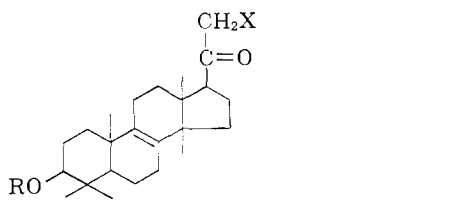
I, R_1 = N, R_2 = CH_2
Ia, R_1 = Ac, R_2 = CH_2
Ib, R_1 = Ac, R_2 = O

II III



IV

V, R = H
Va, R = Ac



VI, R = H, X = OH
VIa, R = Ac, X = OAc
VIb, R = H, X = OAc
VIc, R = Ac, X = OH
VId, R = Ac, X = H
VIe, R = OH, X = H

Acetyl eburicoic acid (Ia)[4] on ozonolysis (1 mole equivalent)[5] in chloroform–ethyl acetate at −70° and decomposition of the ozonide with zinc and acetic acid yielded the keto acid Ib,[6] m.p. 236–238°; $[\alpha]^{23}D$ +52°[7]; λ_{max}^{Nujol} 5.83–5.90, 8.05 μ; calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. Found: C, 74.64; H, 9.54, which on enol lactonization with acetic anhydride and sodium acetate[8] furnished as the major product the endocyclic enol lactone II,[9] m.p. 172–174°; $[\alpha]^{23}D$ +37°; λ_{max}^{Nujol} 5.69, 5.78, 5.93 (weak), 8.05, 12.05, 12.62 and 13.30 μ; calcd. for $C_{32}H_{48}O_4$: C, 77.37; H, 9.73. Found: C, 77.41; H, 9.82; and as a minor product the exocyclic double bond isomer III,[9] m.p. 190–191°; $[\alpha]^{23}D$ +64°, λ_{max}^{Nujol} 5.70, 5.79, 5.97 (weak), 8.08, 11.50, 11.80 and 13.50 μ; found: C, 77.56; H, 9.73, separable by chromatography on neutral alumina. Dehydrogenation of both II and III with 10% Pd/C in boiling *p*-cymene afforded the α-pyrone IV, m.p. 228–229°; $[\alpha]^{23}D$ −114°; λ_{max}^{alc} 305 mμ (ε = 8,900); λ_{max}^{Nujol} 5.79, 5.90, 6.11, 6.35, 8.05, 11.90 and 12.69 μ; n.m.r. doublets centered at τ = 2.85 and 4.05, *J* = 7 c.p.s. (protons at C_{22} and C_{23}); calcd. for $C_{32}H_{46}O_4$: C, 77.69; H, 9.37. Found: C, 77.77; H, 9.43. Reduction of IV with $LiAlH_4$ in THF yielded in an unexpected reaction[10] as the major product the triene V,[11] m.p. 166–168°; $[\alpha]^{23}D$ +68°; λ_{max}^{alc} 244 (mμ (ε = 32,800); λ_{max}^{Nujol} 3.10, 9.71 and 10.40 μ; calcd. for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98; found: C, 81.63; H, 10.91; which on acetylation yielded the diacetate Va, m.p. 131–132°; $[\alpha]^{23}D$ +87°; λ_{max}^{alc} 242 mμ (ε = 32,000); λ_{max}^{Nujol} 5.74, 8.02, 8.19 and 9.86 μ; Calcd. for $C_{34}H_{52}O_4$: C, 77.82; H, 9.99. Found: C, 77.75; H, 10.01. Ozonolysis of V and Va in ethyl acetate at −20° with 2 mole equivalents of ozone yielded, respectively, the 20-keto diol VI, m.p. 220–221°; $[\alpha]^{23}D$ +113°; λ_{max}^{Nujol} 2.80, 5.88 μ; Calcd. for $C_{24}H_{38}O_3$: C, 76.96, H, 10.23. Found: C, 76.85; H, 10.20, and the corresponding diacetate VIa, m.p. 187–189°; $[\alpha]^{23}D$ + 101°; λ_{max}^{Nujol} 5.73 (shoulder), 5.78 and 8.03 μ; calcd. for $C_{28}H_{42}O_5$: C, 73.32; H, 9.23. Found: C, 73.10; H, 9.02, both of which reduce tetrazolium reagent. Monoacetylation of VI gave the 21-acetate VIb, m.p. 191–192°; λ_{max}^{Nujol} 2.71, 5.72, 5.80, 8.1 μ; calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68; found: C, 74.97; H, 9.48, which could be oxidized to the 3-ketone with CrO_3 in acetone, m.p. 168–170°; λ_{max}^{Nujol} 5.71, 5.79, 5.88, 8.09 μ. Hydrolysis of the diacetate VIa with potassium carbonate in methanol furnished the 3-monoacetate VIc, m.p. 202–205°; $[\alpha]^{23}D$ +93°; λ_{max}^{Nujol} 2.83, 5.78, 5.86, 7.98 μ; found: C, 75.14; H, 9.64, which was converted into the 21-desoxy

(4) T. Kariyone and G. Kurono, *J. Pharm. Soc. Japan*, **60**, 110 318 (1940); R. M. Gascoigne, J. S. E. Holker, B. J. Ralph and A. Robertson, *Nature*, **166**, 6526 (1950); F. N. Lahey and P. H. A. Strasser, *J. Chem. Soc.*, 873 (1951).

(5) Over-ozonolysis led to increasing amounts of the 7,9(11)-dehydro derivative of Ib.

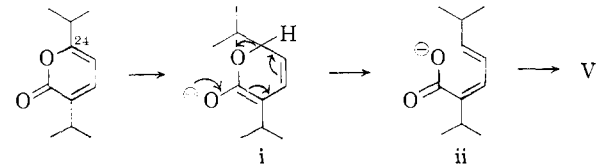
(6) R. M. Gascoigne, J. S. E. Holker, B. J. Ralph and A. Robertson, *J. Chem. Soc.*, 2346 (1951). These workers described the keto acid as an amorphous product without giving analytical data.

(7) All rotations taken in chloroform.

(8) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, *J. Am. Chem. Soc.*, **74**, 4223 (1952).

(9) The position of the enolic double bond was ascertained: Ozonolysis of II and III in CCl_4 and acetic acid, respectively, and by decomposition with water gave, on distillation, isobutyric acid and acetone, respectively, the former identified as its sodium salt by infrared analysis. The n.m.r. spectrum of II showed a single proton resonance centered at τ = 5.17, which is absent in III. Compound III showed 3 protons each at τ = 8.29 and 8.38 $\left(=\!\!<^{CH_3}_{CH_3}\right)$ downfield with regard to the signals for the remaining methyl groups in III, and all methyl groups in II, excepting the $COCH_3$ group.

(10) Of several mechanistic rationalizations for the formation of V, the most reasonable involves an initial hydride attack δ to the carbonyl at C_{24}, stabilization of the resulting intermediate (i) to form the dienoate (ii) which latter is reduced normally to V. This sequence is favored for two reasons:



i ii

(1) The esters and lactones involving the C_{21} carboxyl group of eburicoic acid including the pyrone IV, are unusually resistant to attack by anionic species (steric hindrance) and (2); The δ-carbon atom of α-pyrones exhibits considerable electron deficiency as demonstrated by the ready reaction of methyl coumalate with diazomethane to form the 6-methyl derivative (J. Fried and R. C. Elderfield, *J. Org. Chem.*, **6**, 577 (1941)).

(11) Both the triene V and its diacetate are unstable in the presence of light and air. Storage of the crystalline product leads to lowering of specific absorption in the ultraviolet and of the melting point, as a result of the formation of hydroperoxides.

derivative VId, m.p. 166–167°; $[\alpha]^{23}D$ +107°; λ_{max}^{KBr} 5.79, 5.85, 7.97 μ; via the 21-mesylate (m.p. 109–110°), and 21-iodide (m.p. 146–149°). Hydrolysis of VId with *N* KOH in methanol at room temperature for 20 hours furnished the free alcohol VIe, m.p. 247–249°; λ_{max}^{KBr} 2.83, 5.86 μ, which on oxidation with chromium trioxide in acetone gave 4,4,14α-trimethyl-Δ^8-5α-pregnene-3,20-dione, m.p. 203–204°; $[\alpha]^{23}D$ +136°; λ_{max}^{KBr} 5.85 μ; calcd. for $C_{24}H_{36}O_2$: C, 80.85; H, 10.18. Found: C, 80.74; H, 10.28.

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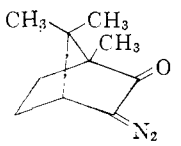
RECEIVED FEBRUARY 19, 1962

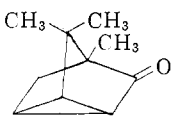
A NOVEL TYPE OF ALKYL SHIFT

Sir:

The formation of cyclocamphanone (II) by the thermal decomposition of diazocamphor (I) has long been known.[1] We have now investigated the thermal decomposition of a 3-diazobicyclo[2.2.1]-

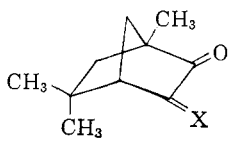


I

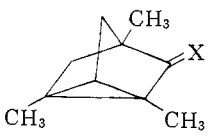


II

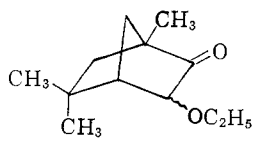
heptan-2-one in which the C_5 atom bears alkyl groups alone. *l*-Isofenchone (III)[2] was converted to its *anti*-isonitroso derivative IV, m.p. 114–114.5°,



III, X = H_2
IV, X = NOH
V = N_2
VI = O



VII, X = O
VIII, X = H_2



IX

$\lambda_{max}^{CCl_4}$ 2.85 (br), 5.76 and 6.06 μ, $[\alpha]D$ (CCl_4) −11.2° (Found: C, 66.24; H, 8.27; N, 7.73), which was converted by the Forster reaction[3] to diazoisofenchone (V), a yellow liquid, $\lambda_{max}^{CCl_4}$ 4.78, 5.89, and 7.49 μ, $[\alpha]D$ (CCl_4) −12.2° (found: C, 67.34; H, 7.95; N, 15.76).

(1) J. Bredt and W. Holz, *J. prakt. Chem.*, [2] **95**, 133 (1917), *cf.* R. Schiff, *Ber.*, **14**, 1375 (1881); A. Angeli, *Gazz. chim. ital.*, **24**, II, 317 (1894.)

(2) O. Wallach, *Ann.*, **362**, 174 (1908); **363**, 1 (1908); D. Mukherji and J. C. Bardhan, *J. Chem. Soc.*, 197 (1949).

(3) M. O. Forster, *ibid.*, **107**, 260 (1915); *cf.* M. P. Cava, R. L. Litle and D. R. Napier, *J. Am. Chem. Soc.*, **80**, 2257 (1958).

Decomposition of V in a 0.06% solution in boiling benzene under nitrogen in the presence of a large excess of copper bronze gave as the major product (average yield, 53%) an optically active, colorless crystalline ketone, $C_{10}H_{14}O$, m.p. 49–50°, $\lambda_{max}^{CCl_4}$ 5.68 μ, $\lambda_{max}^{CS_2}$ 12.05 μ, λ_{max}^{EtOH} 268 mμ (ϵ 71), ϵ_{215} 756 (*cf.* II: $\lambda_{max}^{CCl_4}$ 5.68 μ, $\lambda_{max}^{CS_2}$ 12.20 μ, λ_{max}^{EtOH} 263 mμ (ϵ 68), ϵ_{215} 850), $[\alpha]D$ ($CHCl_3$) −12.8° (Found: C, 80.11; H, 9.47: mol. wt., 171).[4] Its n.m.r. spectrum shows three signals of equal intensity with τ = 8.88, 8.91 and 8.98 p.p.m., attributable to three methyl groups in slightly different environments each attached to a carbon atom which bears no hydrogen atoms,[5] and complex absorption in the region τ = 8.2–8.7 p.p.m., but no signal with τ <8.24 p.p.m. The ketone was converted to its hydrazone, $C_{10}H_{16}N_2$, m.p. 37–41°, $\lambda_{max}^{CCl_4}$ 2.84, 2.91, 5.88 μ, $[\alpha]D$ ($CHCl_3$) −7.9° (Found: C, 73.29; H, 9.91; N, 17.11), which on reduction with sodium ethoxide in ethylene glycol in the presence of hydrazine yielded a hydrocarbon, $C_{10}H_{16}$, b.p. *ca.* 60° (40 mm.), $\lambda_{max}^{CCl_4}$ 3.26 μ (sh), $\lambda_{max}^{CS_2}$ 12.62 μ (found: C, 88.97; H, 11.07; mol. wt., 145). This product is *optically inactive:* α (*c* 4% in CCl_4) <0.05° at 1760, 400, 350, 300 and 270 mμ.[6] Its n.m.r. spectrum shows three singlets with τ = 8.86, 8.90 and 9.04 p.p.m. (intensity ratio 3:6:1); the first two of these signals are assigned to three methyl groups, two of which are now in identical environments, while the third signal can be assigned to a single cyclopropyl hydrogen atom[7]; the spectrum also shows complex absorption in the τ = 8.4–8.6 p.p.m. region, but no signal with τ <8.43 p.p.m. These data establish the structures of the ketone and hydrocarbon as VII and VIII, respectively.[8]

The rate of copper-catalyzed decomposition of V in boiling benzene is appreciably less than that of I under equivalent conditions; semi-quantitative studies indicate that the rate of each reaction is first order in diazo ketone and that the ratio of the rate constants is *ca.* 1:13. Copper-catalyzed decomposition of V in ethanol at 130° gave no VII, but gave a compound (74%), $C_{12}H_{20}O_2$, b.p. 90° (6 mm.), $\lambda_{max}^{CCl_4}$ 5.74, 9.64 μ (Found: C, 73.64; H, 10.38). This was reduced by zinc and acetic acid

(4) A second product (average yield, 15%) from this reaction, $C_{20}H_{28}O$, m.p. 57–60.5°, $\lambda_{max}^{CCl_4}$ 5.69, 5.73 μ (Found: C, 79.97; H, 9.60; mol. wt., 274), was isolated. In the presence of air, *l*-isofenchoquinone (VI) also was formed. When the concentration of the diazo ketone was $\geq$ 1%, the major product obtained was the corresponding azine, m.p. 174–175.5°, $\lambda_{max}^{CHCl_3}$ 5.76 (s), 6.04 (m) μ (found: C, 72.91; H, 8.47; N, 8.59).

(5) An alternative interpretation involving assignment of the signals with τ = 8.88 and 8.98 p.p.m. to two methyl groups in identical environments, each situated on a carbon atom bearing a single hydrogen atom, is excluded on the basis of the spectrum of the related hydrocarbon (*vide infra*).

(6) We thank Mr. G. Holzwarth for assistance in conducting these measurements.

(7) The signal due to the three cyclopropyl hydrogen atoms of tricyclene falls very close to the signals of the methyl groups. The absence of a signal at correspondingly high field in the spectrum of the ketone may be attributed to attachment of the carbonyl group to the cyclopropyl ring: *cf.* C. D. Anderson, Ph.D. Thesis, Harvard University, 1958.

(8) The absence of spin–spin coupling between the cyclopropyl hydrogen atom and the adjacent methylene group in VIII is attributed to unfavorable geometrical factors: *cf.* H. Conroy in "Advances in Organic Chemistry," Vol. II, ed. by R. A. Raphael, E. C. Taylor and H. Wynberg, Interscience Publishers, Inc., New York, N. Y., 1960, p. 265.