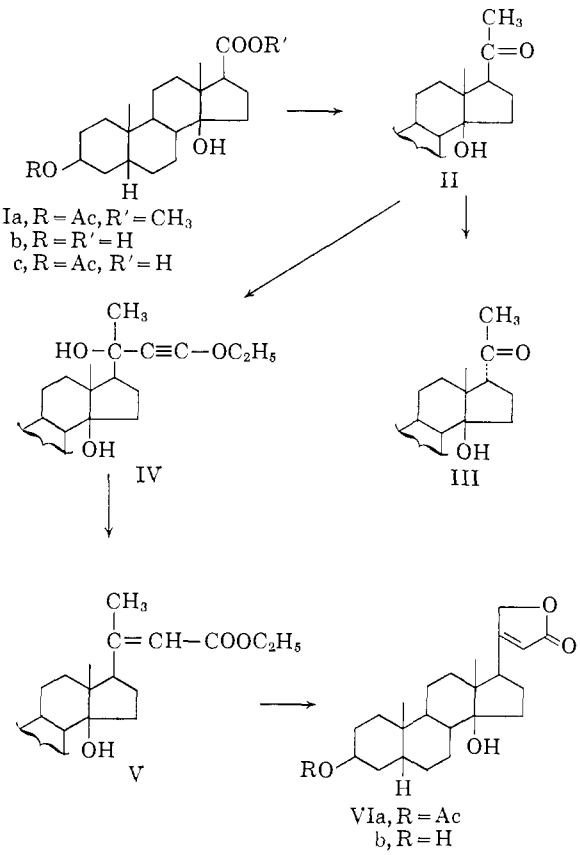

aglycone, which still possesses much of the cardiac activity of the glycosides from which it is derived (*e.g.*, digitoxin, a constituent of digitalis).[2] In this work the main difficulties in building up the natural 14β-hydroxy-17β-butenolide system have been overcome, namely, the ready isomerization of the side chain in 20-keto-C/D-*cis* steroids from the 17β- to the more stable 17α-configuration, the ease of dehydration of the 14β-hydroxyl group with acids and the facility with which this group undergoes reactions with functions in the 17β-side chain.[2,7]

Methyl 3β-acetoxy-14β-hydroxy-5β-etianate (Ia), available by a nine-step sequence from 5β-androstan-3β-ol-17-one acetate,[8] was saponified with potassium carbonate in boiling aqueous methanol to the hydroxy-acid Ib [m.p. 221–223°; $[\alpha]_D$ −9° (EtOH)] and then acetylated to the acetoxy-acid Ic [m.p. 226–228°; $[\alpha]_D$ + 36° ($CHCl_3$)]. Treatment of Ic with an excess of methyllithium in tetrahydrofuran afforded 45% of 5β-pregnane-3β,14β-diol-20-one 3-acetate (II) [m.p. 150–151°; $[\alpha]_D$ + 25° ($CHCl_3$); $\nu_{max}^{CHCl_3}$ 1727 and 1697 cm.$^{-1}$].[9]

The fact that II still possesses a 17β-oriented side chain was demonstrated by its ready inversion through treatment with boiling 3% methanolic potassium hydroxide to give (after re-acetylation) the 17-iso ketone III [m.p. 177–178°; $[\alpha]_D$ −44° ($CHCl_3$); $\nu_{max}^{CHCl_3}$ 1727 and 1712 cm.$^{-1}$]. The lowering in optical rotation on passing from II to III is in keeping with previously made observations regarding the change in rotation when inverting the side chain in 14β-hydroxypregnan-20-ones from 17β to 17α.[10]

Condensation of II with lithium ethoxyacetylide in tetrahydrofuran gave the ethoxyacetylenic carbinol IV ($\nu_{max}^{CHCl_3}$ 2272 cm.$^{-1}$) which was treated with aqueous methanol containing 2% of sulfuric acid for 1 hr. at room temperature. Re-acetylation of the product, followed by purification through repeated chromatography on silica, produced in 45% yield (based on II) the unsaturated ester V [λ_{max}^{EtOH} 232.5 mμ (ϵ 12,000); $\nu_{max}^{CHCl_3}$ 1724 and 1642 cm.$^{-1}$].

Oxidation of V with selenium dioxide under carefully defined conditions (boiling in benzene for 10 hr.) yielded 30% of digitoxigenin acetate (VIa) [m.p. 224–225°, $[\alpha]_D$ + 21° ($CHCl_3$)]. Finally, saponification with 5% hydrochloric acid in methanol (1:1) for 20 hr. at room temperature, or by absorption in ether solution on a column of alkaline alumina for 16 hr., in each case yielded over 80% of digitoxigenin (VIb) [m.p. 246–249°, $[\alpha]_D$ + 19° (EtOH)]. Both VIa and VIb were identified by direct comparison with authentic samples.

(7) Unpublished experiments by Drs. A. Meisels and S. Burstein, to be reported in the full paper.

(8) K. Meyer, *Helv. Chim. Acta*, **29**, 1580 (1946); L. Ruzicka, P. A. Plattner, H. Heusser and K. Meier, *ibid.*, 1342 (1947).

(9) This reaction was based on the corresponding one in the 5α-series carried out previously in these Laboratories by Dr. A. Meisels.

(10) P. A. Plattner, L. Ruzicka, H. Heusser and E. Angliker, *Helv. Chim. Acta*, **30**, 385 (1947); H. Hasegawa, Y. Sato and K. Tsuda, *Chem. Pharm. Bull.* (Japan), **9**, 409 (1961).

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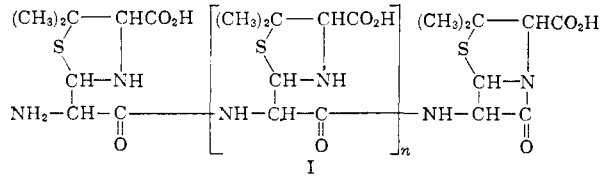
POLY-6-AMINOPENICILLANIC ACID

Sir:

The β-lactam of 6-aminopenicillanic acid (6-APA) is susceptible to cleavage by bases and penicillinase, giving an α-amino acid (penicic acid) as the product. Our study of facile catalytic β-lactam cleavage of 6-APA and penicillins in frozen systems showed that ring opening in 6-APA can occur in the absence of added catalyst.[1] Further study indicates that under various conditions polymerization to low molecular weight peptides accompanies ring opening in potassium 6-APA solutions.

The reaction apparently consists in nucleophilic attack by the primary amino group on the β-lactam of a neighboring molecule, forming polyamides of various chain lengths having the probable structure I.



I

The reaction has been observed in K 6-APA solutions in water (pH 6–7) or phosphate buffer,

(1) N. H. Grant, D. E. Clark and H. E. Alburn, *J. Am. Chem. Soc.*, **83**, 4476 (1961).

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 $C_8H_{11}O_8N_2SK$: 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.

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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.