COMMUNICATIONS TO THE EDITOR

BIOSYNTHESIS OF L-GULONIC ACID IN RATS AND GUINEA PIGS

Sir:

The most likely pathway for the biosynthesis of L-ascorbic acid in rats is D-glucose → D-glucuronolactone → L-gulonolactone → L-ascorbic acid.[1-5] Unlike rats, guinea pigs are unable to convert L-gulonolactone to L-ascorbic acid, which may explain their inability to synthesize the vitamin.[6,7] In both species, administered L-gulonolactone is extensively oxidized to CO_2,[5] and a small fraction of the dose is excreted in urine as L-gulonic acid.[8] No evidence has been presented, however, for the biosynthesis and occurrence of L-gulonic acid or its lactone in animal species. The present studies show that D-glucose-1-C^{14} and D-glucuronolactone-6-C^{14} are converted *in vivo* to labeled L-gulonic acid.

The method for the isolation of labeled L-gulonic acid from urine is given[9]: 300 mg. of non-radioactive L-gulonic acid was added to a 24-hour urine sample which was passed through an Amberlite IR-4B column in the acetate form.[10] The adsorbed L-gulonic acid was eluted with 2 *N* formic acid and the eluate was evaporated rapidly to dryness *in vacuo* at 50°. L-Gulonic acid was converted to its lactone by crystallization from glacial acetic acid. The resulting L-gulonolactone was dissolved in water and the solution was passed through the Amberlite IR-4B column.[10] L-Gulonolactone was obtained after evaporation of the effluent to dryness, and its radioactive purity was established by finding constant specific activity on successive recrystallizations from glacial acetic acid and absolute ethanol. In addition, two derivatives, the potassium acid saccharate and the phenylhydrazide, prepared from the same sample of L-gulonolactone, had identical molar specific activities. Control experiments carried out by adding either D-gluconic acid-1-C^{14} or D-glucuronic acid-6-C^{14} to non-radioactive urine showed that these compounds did not contaminate the isolated L-gulonolactone.

D-Glucuronolactone-6-C^{14} was administered to rats[11] and guinea pigs in intraperitoneal doses of 20 mg. (0.5 μc./mg.) and labeled L-gulonic acid was isolated from urine collected over 24 hours. The results obtained showed that both species can convert D-glucuronolactone to L-gulonic acid the % conversion being 0.72 and 2.4 in two rats and 0.90, 1.3 and 2.9 in three guinea pigs.

The conversion of D-glucose-1-C^{14} to urinary L-gulonic acid was measured in rats[11] receiving either Chloretone or barbital to stimulate the synthesis of L-ascorbic acid[12] (Table I). Similar experiments also carried out in rats[11] not receiving drugs.

TABLE I

CONVERSION OF D-GLUCOSE-1-C^{14} TO URINARY L-GULONIC ACID IN RATS[a]

Drug	None		Chloretone[b]		Barbital[b]	
Conversion, %	0.04	0.03	0.34	0.54	0.22	0.16

[a] Urine was collected for 24 hours after 10 to 30 mg. intraperitoneal doses of D-glucose-1-C^{14} (1.0 μc./mg.). [b] Rats were fed daily either 150 mg. of barbital or 50 mg. of Chloretone for at least 5 days prior to the experiment.

It will be noted that the conversion of D-glucose-1-C^{14} to urinary L-gulonic acid averaged 0.3% in drug-treated rats, but no conversion was detected in animals not receiving drugs (<0.03%). Administration of Chloretone and barbital has been found also to produce in rats a similar increase in conversion of D-glucose-1-C^{14} to urinary D-glucuronic acid and L-ascorbic acid.[8] The possible mechanism by which drugs exert this effect on the formation of D-glucuronic acid, L-gulonic acid and L-ascorbic acid is now under investigation.

(1) H. H. Horowitz and C. G. King, *J. Biol. Chem.*, **200**, 125 (1953).
(2) J. J. Burns and E. H. Mosbach, *ibid.*, **221**, 107 (1956).
(3) H. H. Horowitz and C. G. King, *ibid.*, **205**, 815 (1953).
(4) F. A. Isherwood, T. Y. Chen and L. W. Mapson, *Biochem. J.*, **56**, 1 (1954).
(5) J. J. Burns and C. Evans, *J. Biol. Chem.*, **223**, 897 (1956).
(6) J. J. Burns, P. Peyser and A. Moltz, *Science*, **124**, 1148 (1956).
(7) M. U. Hassan and A. L. Lehninger, *J. Biol. Chem.*, **223**, 123 (1956).
(8) J. J. Burns, C. Evans and P. G. Dayton, unpublished observations.
(9) The methods for preparation and assay of samples for C^{14} were the same as those used previously.[3] D-Glucose-1-C^{14} and D-glucuronolactone-6-C^{14} were obtained from the National Bureau of Standards, Washington, D. C.
(10) J. J. Burns, E. H. Mosbach, S. Schulenberg and J. Reichenthal, *J. Biol. Chem.*, **214**, 507 (1955).
(11) Wistar strain.
(12) H. E. Longenecker, H. H. Fricke and C. G. King, *J. Biol. Chem.*, **135**, 497 (1940).

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A NEW METHOD FOR DEHYDROGENATION OF CORTICOSTEROIDS

Sir:

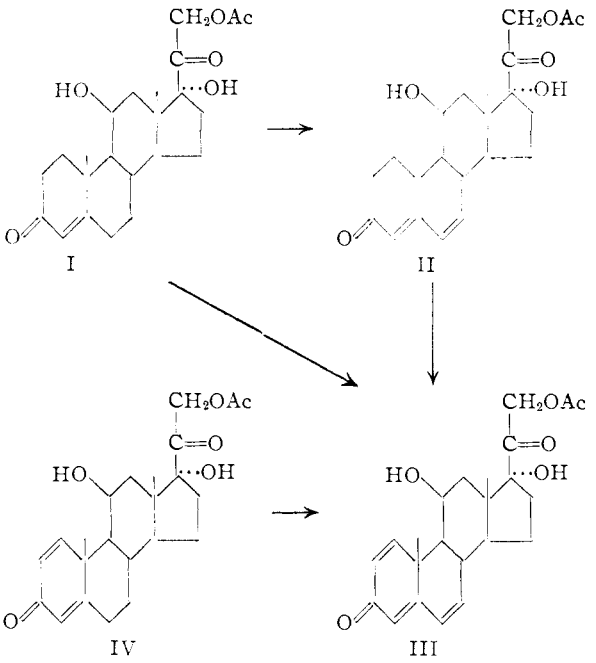
We wish to report a new, single-step method for the synthesis of unsaturated analogs of Δ^4-3-ketosteroids. It has been found that a variety of steroid Δ^4-3-ketones can be selectively oxidized with chloranil under mild conditions to Δ^6-dehydro derivatives. Detailed studies in the hydrocortisone series have revealed that more vigorous reaction conditions result in the formation of the corresponding $\Delta^{1,4,6}$-trienone derivative. The $\Delta^{1,4,6}$-3-one function is a previously unreported structural modification of glucocorticoids.

Hydrocortisone acetate (I), when treated with chloranil in refluxing xylene, yielded 66% of $\Delta^{4,6}$-pregnadiene-11β,17α,21-triol-3,20-dione acetate (II), m.p. 204.0–205.0°, $[\alpha]^{25}D$ +199° (dioxane), λ_{max}^{alc} 284 mμ (25,000); *Anal.* Calcd. for C_{23}-

$H_{30}O_6$: C, 68.6; H, 7.51. Found: C, 68.6; H, 7.52. II was convertible to the known $\Delta^{4,6}$-pregnadiene-17α,21-diol-3,11,20-trione acetate by chromic acid oxidation.

When I was treated with chloranil in refluxing *n*-amyl alcohol, the major product[1] was $\Delta^{1,4,6}$-pregnatriene-11β,17α,21-triol-3,20-dione acetate (III),[2] m.p. 210.1–211.3°, $[\alpha]^{24}D$ +131° (dioxane), λ_{max}^{alc} 223 mμ (13,400), 253 mμ (10,500), 301 mμ (13,300).[3] *Anal.* Calcd. for $C_{23}H_{28}O_6$: C, 69.0; H, 7.05. Found: C, 69.3; H, 7.12. The structure of III was confirmed by two independent syntheses: (a) from II by dehydrogenation with chloranil or selenium dioxide[4] and (b) from prednisolone acetate (IV) by dehydrogenation with chloranil. Compound III has been found in animals to be a potent glucocorticoid.[5]

Under conditions analogous to those used in the preparation of II, a number of Δ^6-dehydro steroids



(some of them not attainable by conventional methods) have been prepared, for example: $\Delta^{4,6}$-pregnadiene-17α,21-diol-3,11,20-trione acetate[6] (45% yield), m.p. 233.3–235.8°, $[\alpha]^{25}D$ +265° (dioxane), λ_{max}^{alc} 281 mμ (25,400); $\Delta^{4,6}$-pregnadiene-17α,21-diol-3,20-dione acetate[7] (47% yield), m.p. 221.4–223.7°, $[\alpha]^{25}D$ +112° ($CHCl_3$), λ_{max}^{alc} 283 mμ (22,500); $\Delta^{4,6}$-pregnadiene-11β,14α,17α,21-tetrol-3,20-dione acetate (Δ^6-dehydro-14α-hydroxyhydrocortisone acetate) (50% yield), m.p. 245.3–247.1°, $[\alpha]^{24}D$ +230° (dioxane), λ_{max}^{alc} 283 mu (24,800). *Anal.* Calcd. for $C_{23}H_{30}O_7$: C, 66.0; H, 7.23. Found: C, 65.4; H, 7.20; and $\Delta^{4,6}$-pregnadiene-14α,17α,21-triol-3,11,20-trione acetate (Δ^6-dehydro-14α-hydroxycortisone acetate) (25% yield), m.p. above 260°, $[\alpha]^{20}D$ +292° (dioxane), λ_{max}^{alc} 282 mμ (24,300). *Anal.* Calcd for $C_{23}H_{28}O_7$: C, 66.3; H, 6.78. Found: C, 66.6; H, 6.89.

After this communication was submitted for publication, the synthesis of III by another route was reported by D. Gould, *et al.*, THIS JOURNAL, **79**, 502 (1957).

Details of the method and synthesis of related compounds will be reported in a subsequent communication.

(1) The reaction proceeds through the initial formation of Δ^6-dehydrohydrocortisone acetate (II), which is the major product when a lower ratio of chloranil to steroid is used.

(2) Saponification of III by conventional methods afforded $\Delta^{1,4,6}$-pregnatriene-11β,17α,21-triol-3,20-dione, m.p. 232.8–234.2°, $[\alpha]^{24}D$ +114° (dioxane). λ_{max}^{alc} 221 mμ (11,500), 255 mμ (9,300), 298 mμ (12,400). *Anal.* Calcd. for $C_{21}H_{26}O_5$: C, 70.4; H, 7.31. Found: C, 70.1; H, 7.32. Oxidation of III with chromic acid yielded $\Delta^{1,4,6}$-pregnatriene-17α,21-diol-3,11,20-trione acetate, m.p. 222.5–226.2°, $[\alpha]^{20}D$ +284° (dioxane). λ_{max}^{alc} 223 mμ (10,700), 255 mμ (9800), 297 mμ (12,100). *Anal.* Calcd. for $C_{23}H_{28}O_6$: C, 69.3; H, 6.58. Found: C, 69.3; H, 6.47.

(3) $\Delta^{1,4,6}$-Triene-3-ones are reported to exhibit λ_{max} 223 mμ, 256 mμ and 298 mμ; L. Dorfman, *Chem. Rev.*, **53**, 70 (1953).

(4) During the course of a broader study of dehydrogenation techniques in this laboratory it was found, as others have already described (ref. 4a, b, c, d), that selenium dioxide effects dehydrogenation of a variety of 3-ketosteroids to yield $\Delta^{1,4}$-diene-3-ketones. However, the conversion of a $\Delta^{4,6}$-3-ketosteroid to a $\Delta^{1,4,6}$-triene-3-one derivative has not been reported previously. (a) K. Florey and A. R. Restivo, Abstracts of Papers, Delaware Valley Regional Meeting, Feb. 16, 1956. (b) H. Ringold, *et al.*, *J. Org. Chem.*, **21**, 239 (1956). (c) Ch. Meystre, *et al.*, *Helv. Chim. Acta*, **39**, 734 (1956). (d) S. A. Szpilfogel, *et al.*, *Rec. Trav. Chim.*, **75**, 475 (1956).

(5) The results of the animal tests, which were performed by Dr. R. I. Dorfman of the Worcester Foundation for Experimental Biology, will be reported in another communication.

(6) V. R. Mattox, *et al.*, *J. Biol. Chem.*, **197**, 261 (1952), report m.p. 236–237°, $[\alpha]D$ +243° (acetone), λ_{max}^{alc} 280 mμ (26,000).

(7) F. Sondheimer, *et al.*, THIS JOURNAL, **75**, 5392 (1953), report m.p. 220–222°, $[\alpha]^{20}D$ +104° ($CHCl_3$), λ_{max}^{alc} 284 mμ (log ϵ 4.47).

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GENERAL ACID-BASE CATALYSIS IN THE INTRAMOLECULAR HYDROLYSIS OF PHTHALAMIC ACID[1]

Sir:

It has been shown recently that imidazole, which has been postulated to be a constituent of the active site of hydrolytic enzymes, catalyzes the hydrolysis of some substrates of α-chymotrypsin but is markedly less effective than the enzyme.[2,3] Enzymatic processes proceed through the formation of an adsorptive complex between substrate and enzyme, followed by a catalytic process during which the substrate is constrained with respect to the reactive site. Such constraint likens enzymatic action to intramolecular catalysis, and like many intramolecular reactions in organic chemistry, enzymatic catalysis should proceed at a greater rate than the corresponding intermolecular process.[4]

To test this hypothesis, the hydrolysis of phthalamic acid was investigated.[5] The infrared spec-

(1) This research was supported by Grant H-2416 of the National Institutes of Health. Paper VIII of the series, "The Mechanism of Enzymatic Hydrolysis."

(2) T. C. Bruice and G. L. Schmir, *Arch. Biochem. Biophys.*, **63**, 484 (1956); THIS JOURNAL, **79**, April (1957).

(3) M. L. Bender and B. W. Turnquest, *ibid.*, **79**, April (1957).

(4) This statement implies that imidazole is the sole agent in enzymatic hydrolysis. While there is no question of its participation in enzymatic catalysis, it also appears that the side chain of serine is a participant. See H. Gutfreund and J. S. Sturtevant, *Biochem. J.*, **63**, 656 (1956), and G. H. Dixon and H. Neurath, *J. Biol. Chem.*, in press (1957).

(5) O. Aschan, *Ber.*, **19**, 1402 (1886); E. Chapman and H. Stephens, *J. Chem. Soc.*, **127**, 1793 (1925).