by the coloration with ferric chloride, the ultraviolet spectral shift (λ_{max}^{EtOH} 240 mμ, log ϵ 3.90; $\lambda_{max}^{0.01N\ KOH}$ 274 mμ, log ϵ 4.03) and the formation of an enol acetate VII (m.p. 149–151°, λ_{max}^{EtOH} 218 mμ, log ϵ 4.02; found for $C_{17}H_{22}O_6$: C, 63.46; H, 6.72; acetyl, 13.75), reconvertible to VI by base. That an internal acetal was indeed produced between the initially formed aldehyde grouping and the suitably situated 1,3-glycol system is confirmed by the ozonization of acetylidene iresin, in which the free hydroxyl groups are blocked and which produced the aldehyde VIII (m.p. 222–225°, λ_{max}^{EtOH} 233 mμ, $\lambda_{max}^{0.01N\ KOH}$ 272 mμ, positive Tollens and ferric chloride reactions; found: C, 62.76; H, 7.47). Acid treatment of the aldehyde VIII regenerated the internal acetal VI.

In the light of the above experiments, two expressions (II or isomer with angular methyl group at C-5) present themselves as likely alternatives for iresin. We favor II, a structure following both the isoprene and farnesol rules, on the basis of biogenetic analogy to the higher terpenes. The details of the experimental evidence and the arguments leading to the stereochemistry implicit in structure II will be presented in a complete article.

This represents part of a joint research program, financed by the Rockefeller Foundation, on Latin-American plant products between Wayne University and the National University of Mexico.

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ENZYMATIC OXIDATION OF URIDINE DIPHOSPHATE GLUCOSE TO URIDINE DIPHOSPHATE GLUCURONIC ACID

Sirs:

The synthesis of glucuronides in a cell-free preparation from liver has been shown to require a substance identified as uridine diphosphate glucuronic acid (UDPGA).[1,2] Previous studies have brought evidence against the existence of a biosynthetic pathway of UDPGA from either α- or β-1-phosphoglucuronic acid and uridine triphosphate (UTP),[2] a mechanism which operates in the synthesis of uridine diphosphate glucose (UDPG).[3] These observations suggested that the biosynthesis of glucuronic acid might occur via the oxidation of glucose bound to the uridine nucleotide. We have now found that the particle-free supernatant fluid from liver of several animals oxidizes UDPG to UDPGA in the presence of diphosphopyridine nucleotide (DPN^+), and the enzyme or enzymes which catalyze this oxidation have been purified from liver acetone powder.

When the supernatant fluid from liver homogenate, containing both microsomes and soluble enzymes, was incubated with UDPG,[4,5] DPN^+ and a suitable acceptor (*o*-aminophenol or morphine), the formation of glucuronide could be demonstrated. Further experiments indicated that the UDPG dehydrogenase system was in the particle-free supernatant while the glucuronide coupling enzyme was associated with the microsomes (Table I, Expt. 1).

TABLE I

ENZYMATIC SYNTHESIS OF GLUCURONIDES FROM UDPG

Complete System: Experiment 1: 3.5 ml. 0.014 *M* phosphate buffer, *p*H 7.4, containing 1 μM UDPG, 0.2 μM DPN, 0.5 μM *o*-aminophenol (in 0.01 *M* ascorbic acid), 50 μM $MgCl_2$, washed microsomes from 0.5 g. and particle-free supernatant from 0.1 g. wet guinea pig liver; Experiment 2: Fractionated enzymes employed with DPN (4 μM) in excess over UDPG (1 μM) (*cf.* footnote *b*)).

	Optical density, 535 mμ[a] Experiment 1	Experiment 2
Complete	470	...
Minus DPN^+	110	...
Minus UDPG	125	...
Minus microsomes	0	20
Minus particle-free supernatant	10	...
Preincubation[b]	340	720
Preincubation minus DPN^+	...	25
Preincubation with semicarbazide[c]	...	720

[a] *o*-Aminophenol glucuronide was measured by the procedure of G. A. Levvy and I. D. E. Storey (*Biochem. J.*, **44**, 295 (1949)). Similar results were obtained using morphine as the acceptor, measured by a modification of the method of F. E. Shideman and A. R. Kelley (*Science*, **106**, 298 (1947)). [b] UDPG and DPN^+ were incubated with particle-free supernatant (Expts. 1) or with purified enzyme (Expt. 2). At the completion of the reaction, the mixture was boiled, and the filtrate was then incubated with washed microsomes and *o*-aminophenol. [c] As in *b*, except that the preincubation was carried out in 0.03 *M* semicarbazide.

The enzyme or enzymes which catalyze this oxidation have been purified 180-fold in 40% yield from a water extract of calf liver acetone powder. Uridine diphosphate acetylglucosamine,[6] α-glucose-1-phosphate and ethyl alcohol are not oxidized by this preparation. With the purified enzyme more than 90% of the added UDPG was oxidized and for each mole oxidized 2 moles of DPN^+ were reduced (Fig. 1).

The following evidence has been obtained that the product of these two oxidations is UDPGA and, therefore, that the sole target of the two oxidations is the 6 position of the glucose bound to UDPG. The product of the enzymatic oxidation, adsorbed on charcoal and eluted with 50% ethanol, gave a positive carbazole color reaction for glucuronic acid with a maximum adsorption of 525 mμ.[7] A larger digest was chromatographed on a Dowex 1-Cl

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(5) Our thanks are due to Dr. J. C. Keresztesy and Mr. H. Lutterlough of this institute for assistance with a large scale preparation of UDPG (*cf.* ref. 6).

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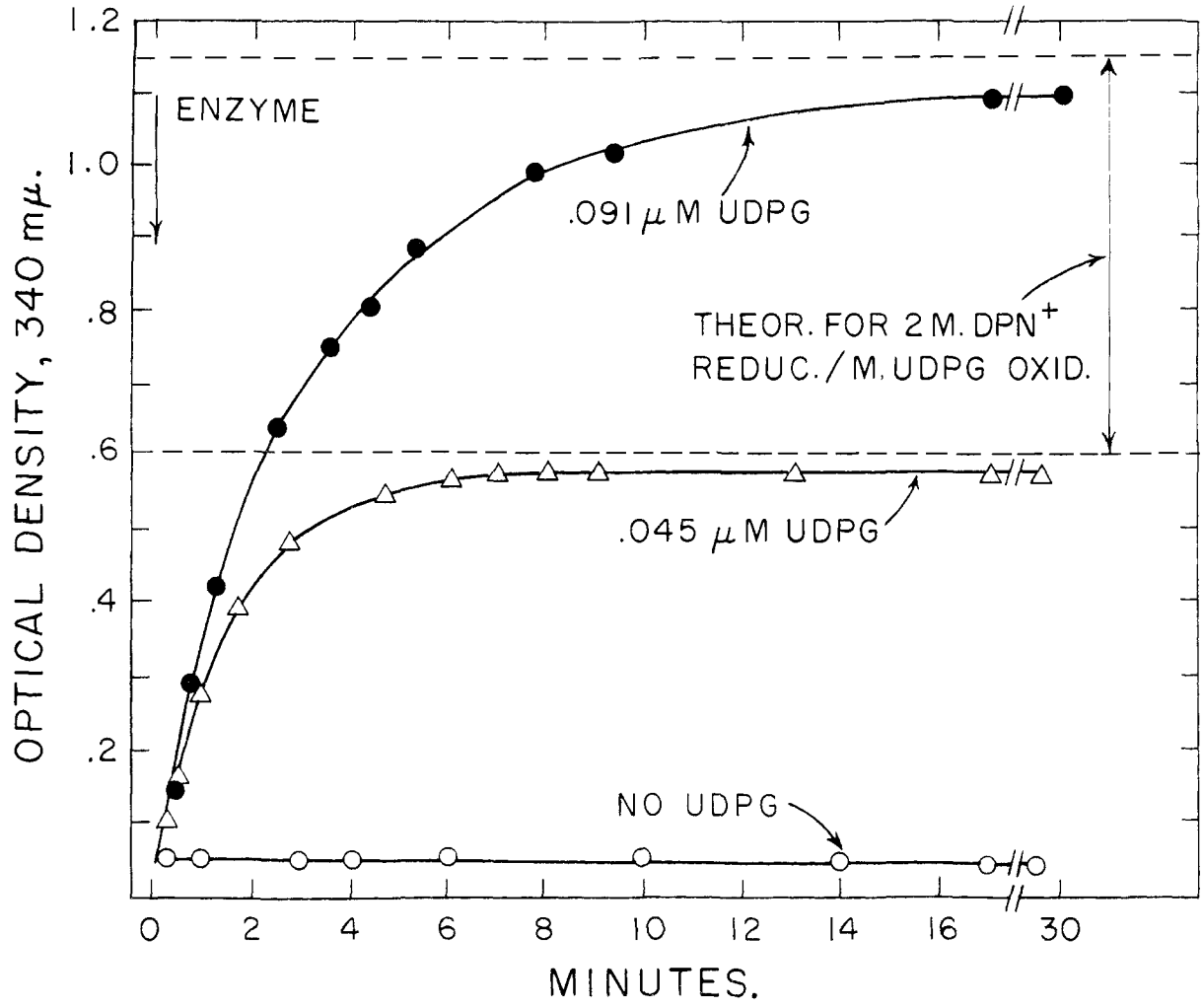

Fig. 1.—Reduction of DPN^+ by purified UDPG dehydrogenase. Each cuvette contained 0.1 *M* diethanolamine buffer, *p*H 9.0, 0.4 μM DPN^+, UDPG as indicated and enzyme in 1 cc.

column and eluted with an NaCl gradient in 0.01 *N* HCl. A zone in the region expected for UDPGA contained a UDP compound which gave carbazole color. For each mole of uridine (ultraviolet adsorption) 0.93 mole of glucuronic acid was present, based on the carbazole reaction. The enzymatic oxidation product of UDPG, even when generated in the presence of semicarbazide as an aldehyde trap, could be utilized to generate *o*-aminophenol glucuronide in the presence of washed microsomes and *o*-aminophenol (Table I). At least 75% of the generated UDPGA could be transferred to the acceptor. No evidence for the appearance of an intermediate compound at the oxidation level of aldehyde has so far been obtained; attempts to accumulate such a compound are in progress.

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THE PARTIAL SYNTHESIS OF TOMATIDINE[1]

Sir:

The steroid alkaloid tomatidine,[2] derived by hydrolytic cleavage of the tetroside tomatine, native to certain species of *Lycopersicum*, has been shown to yield as important degradation fragments Δ^{16}-allopregnen-3β-ol-20-one[3] and tigogenin lactone.[4] These scission products, together with the secondary nature of the alkamine, diagnostic behavior under conditions of hydrogenation, and empirical composition $C_{27}H_{45}NO_2$, have supported the attribution of a skeletal formulation akin to that characteristic of solasodine, the structure of which has been confirmed by partial synthesis from kryptogenin[5] and from diosgenin.[6] Inasmuch as it is now known[7] that diosgenin and sarsasapogenin give rise,

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