tra of the pyridine hemochromogens prepared from the two hemins were also identical, having an absorption peak in the oxidized form at 390 mμ, and peaks in the reduced form at 407, 518 and 547 mμ.

Some years ago Keilin and Hartree[6] observed that when reduced cytochrome *c* was cooled to liquid nitrogen temperature in a vitrified solvent, the usual α-band was resolved into two bands. Since the cytochrome *c* employed was less pure than that now available, it seems possible in retrospect that the phenomenon was due to contaminating hemoproteins. In view of the fact that two hemins can be derived from more highly purified cytochrome *c*, it was of interest to determine whether the latter would also show a split α-band at low temperature. When this cytochrome *c* (reduced) was "vitrified" by the addition of two volumes of glycerol and cooled in liquid nitrogen, a spectrum with two bands was observed, with the strongest absorption at approximately 548 mμ and a weaker but distinct absorption band at a slightly lower wave length.

The results reported thus indicate that highly purified cytochrome *c* consists of two hemoproteins which yield chromatographically distinct hematohemins. The physical separation and study of the catalytic properties of the hemoproteins from which these hemins can be derived are under investigation.

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(6) D. Keilin and E. F. Hartree, *Nature*, **164**, 254 (1949).

DEPARTMENT OF BIOCHEMISTRY M. MORRISON
THE UNIVERSITY OF ROCHESTER R. W. ESTABROOK
SCHOOL OF MEDICINE AND DENTISTRY E. STOTZ
ROCHESTER, NEW YORK

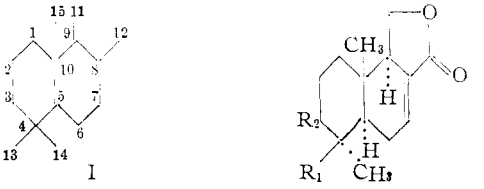
RECEIVED NOVEMBER 23, 1954

TERPENOIDS XV.[1] THE CONSTITUTION OF IRESIN. A NEW FUNDAMENTAL SESQUITERPENE SKELETON.

Sir:

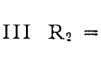
The fact that the bicyclofarnesol skeleton I,[2] present in all cyclic di- and tri-terpenes, has never been encountered among sesquiterpenes, has led to the suggestion by Ruzicka[3] in an outstanding review on the biogenesis of terpenes that "*this appears to indicate that the biogenesis of steroids, diterpenes and triterpenes differs in some fundamental detail from that of the monoterpenes and sesquiterpenes.*" We should now like to propose that the recently discovered[4] sesquiterpene iresin ($C_{15}H_{22}O_4$) represents the long sought-after "missing link" between the lower and higher terpenes since it appears to be based on such a skeleton (I).

Iresin possesses a 1,3-glycol system since it is unaffected by lead tetraacetate but can be transformed into a benzylidene (m.p. 242–244°) or acetylidene derivative (m.p. 283°; found: C, 69.96; H, 8.25). The nature of the basic ring system was established by the palladium dehydrogenation which furnished 1,5-dimethylnaphthalene and 1,5-dimethyl-2-hydroxynaphthalene[5] (m.p. 163.5–164.5°; found: C, 83.74; H, 7.00. Benzoate, m.p. 151.5–153°; found: C, 82.29; H, 5.99). Since the hydroxyl group surviving in the dehydrogenation product cannot have been incorporated[6] in the α,β-unsaturated butenolide moiety[4] of iresin, this automatically limits the placement of the remaining alcoholic function to C-1 or C-13. The latter position as well as the presence of the C-14 methyl group could be proved rigorously in the following manner.[7]



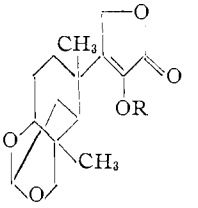
I

II R_2 =  ; $R_1 = CH_2OH$

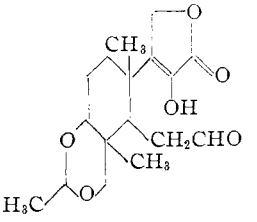
III R_2 =  ; $R_1 = CH_2OC(C_6H_5)_3$

IV $R_2 = O$; $R_1 = CH_2OC(C_6H_5)_3$

V $R_2 = O$; $R_1 = H$



VI R = H
VII R = Ac



VIII

Conversion of iresin (II) to the 13-monotrityl ether (III) (m.p. 258–260°; found: C, 80.74; H, 7.13) followed by chromium trioxide–pyridine oxidation to the 3-keto-13-trityl ether (IV) (m.p. 295–298°; found: C, 80.92; H, 7.01) and acid hydrolysis yielded formaldehyde[8] and the corresponding 13-nor-3-ketone (V) (m.p. 146–148°, $[\alpha]$ + 74°, λ_{max}^{EtOH} 224 mμ, log ε 4.11, $\lambda_{max}^{CHCl_3}$ 5.68 (lactone), 5.85 (ketone) and 5.92 μ (double bond)[4]; found: C, 71.87; H, 7.76). Ozonization of iresin (II) yielded VI (m.p. 230–233°, $[\alpha]^{23}D$ −26°, $\lambda_{max}^{CHCl_3}$ 2.82, 3.00, 5.69 and 5.95 μ (double bond)[4]; found for $C_{15}H_{20}O_5$: C, 64.22; H, 7.06); the presence of the enol lactone grouping is indicated

(1) Paper XIV, C. Djerassi, G. H. Thomas and O. Jeger, *Helv. Chim. Acta*, **38**, in press (1955).

(2) We are employing a numbering system which, as far as possible, is analogous to that of the steroids and triterpenes.

(3) L. Ruzicka, *Experientia*, **9**, 357 (1953).

(4) C. Djerassi, P. Sengupta, J. Herran and F. Walls, THIS JOURNAL, **76**, 2966 (1954).

(5) L. Ruzicka and E. Rey, *Helv. Chim. Acta*, **26**, 2136 (1943). We are indebted to Dr. O. Jeger (Zurich) for an authentic sample of the benzoate from which the naphthol was regenerated.

(6) The fact that iresin is recovered unchanged (after acidification) after treatment with strong base (potassium *t*-butoxide) indicates that the double bond is exocyclic to the lactone ring. This requirement and the ozonization experiments eliminate the possibility that the C-3 hydroxyl group is involved in the lactone ring.

(7) An alternate proof, to be described in the detailed paper, involves the preparation and reactions of the 3-keto-13-formyl derivative (negative ferric chloride reaction).

(8) A similar retro-aldol reaction has been described with icterogenin (D. H. R. Barton and P. deMayo, *J. Chem. Soc.*, 887 (1953)).

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.

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SAMUEL C. HOOKER LABORATORY
DEPARTMENT OF CHEMISTRY
WAYNE UNIVERSITY
DETROIT, MICHIGAN, AND
INSTITUTO DE QUIMICA
UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO
MEXICO, D.F.

CARL DJERASSI
W. RITTEL[9a]
A. L. NUSSBAUM[9b]
F. W. DONOVAN[9c]
J. HERRAN

RECEIVED NOVEMBER 10, 1954

(9) (a) Postdoctorate Research Fellow, 1953–1954; (b) U. S. Public Health Service Predoctorate Research Fellow, 1952–1954; (c) Postdoctorate Research Fellow, 1954–1955.

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

(1) G. J. Dutton and I. D. E. Storey, *Biochem. J.*, **58**, xxvii (1953); **57**, 275 (1954).

(2) E. E. B. Smith and G. T. Mills, *Biochem. Biophys. Acta*, **13**, 386 (1954).

(3) A. Munch-Petersen, H. M. Kalckar, E. Cutolo and E. E. B. Smith, *Nature*, **172**, 1036 (1953).

(4) R. Caputto, L. F. Leloir, C. E. Cardini and A. C. Paladini, *J. Biol. Chem.*, **184**, 333 (1950).

(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).

(6) E. Cabib, L. F. Leloir and C. E. Cardini, *J. Biol. Chem.*, **203**, 1055 (1953).

(7) Z. Dische, *ibid.*, **183**, 489 (1950).