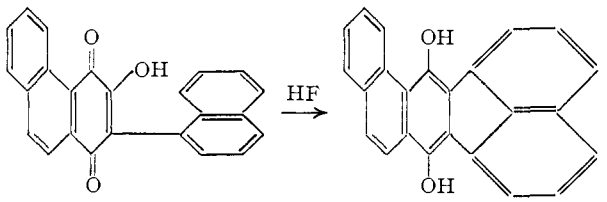
tablished by analysis, by its lack of color and by the fact that it forms a diacetate. Apparently, stabilization of the hydroquinone II relative to the corresponding quinone results from fusion of the highly strained five-membered ring to the quinoid center. That fused five-membered rings do indeed increase the oxidation potentials of such quinoid systems has been noted by a number of investigators.[2-5]



I II

Experimental

7,14-Dihydroxyacenaphtho[1,2-b]phenanthrene (II).—One gram of 3-hydroxy-2-(α-naphthyl)-1,4-phenanthrenequinone (I)[1] was added to 80 g. of anhydrous hydrogen fluoride in a copper alloy container. After stirring well with a copper wire, a tight fitting closure was screwed to the container. After standing at room temperature overnight, the mixture was poured into ice and the product was taken up in ether, washed successively with water, sodium bicarbonate solution, and more water, and then dried over anhydrous magnesium sulfate. Filtration, removal of the ether by distillation and trituration of the residue with pentane gave 0.7 g. of crude material which after two recrystallizations from benzene (charcoal treatment was included in the first crystallization) gave nearly colorless crystals, m.p. 175–177°.

Anal. Calcd. for $C_{24}H_{14}O_2$: C, 86.21; H, 4.22. Found: C, 86.21; H, 4.59.

The product is insoluble in aqueous sodium bicarbonate but dissolves in a large volume of aqueous potassium hydroxide.

7,14-Diacetoxyacenaphtho[1,2-b]phenanthrene.—A solution of 0.5 g. of crude II in 10 cc. of acetic anhydride containing 0.5 g. of anhydrous potassium acetate and a few pieces of 20-mesh zinc was heated on the steam-bath for 45 minutes. After pouring into water and standing overnight the solid product remaining was collected by filtration and dried. The 0.6 g. of crude material obtained in this way was recrystallized three times from heptane to give the pure diacetate, m.p. 180–182°.

Anal. Calcd. for $C_{28}H_{18}O_4$: C, 80.37; H, 4.34. Found: C, 80.39; H, 4.89.

Acknowledgment.—The author is grateful to Mr. E. F. Shelberg for the microanalyses.

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(2) L. F. Fieser, THIS JOURNAL, **50**, 439 (1928).
(3) R. T. Arnold and H. E. Zaugg, *ibid.*, **63**, 1317 (1941).
(4) C. F. Koelsch and E. J. Prill, *ibid.*, **67**, 1296 (1945).
(5) T. Posternak and R. Castro, *Helv. Chim. Acta*, **31**, 536 (1948).

COMMUNICATIONS TO THE EDITOR

MULTIPLE PROSTHETIC GROUPS IN CYTOCHROME C

Sir:

The well known cytochrome *c* preparation of Keilin and Hartree[1] has been purified further by Margoliash,[2] who employed a column chromatographic procedure which appears to eliminate both a non-cytochrome *c* hemoprotein and an enzymatically inactive cytochrome *c*. In spite of the high degree of purity of the product and its homogeneity on the column employed, observations recorded in this paper demonstrate that two closely related hematohemins may be derived from the preparation and that they probably represent two molecular species of cytochrome *c*.

The "enzymatically active" fraction of cytochrome *c* prepared by the Margoliash procedure was subjected to the silver treatment of Paul[3] for the removal of hemin. The hemin solution so obtained was evaporated to dryness, dissolved in chloroform, and chromatographed on the silicic acid column described by Morrison and Stotz.[4] Eluent from the column was distributed in the tubes of an automatic fraction collector and the concentration of hemin in each aliquot was determined by its optical density at 410 mμ. The resulting chromatogram as illustrated shows two distinct peaks, representing two hemins derived from the purified cytochrome *c*.

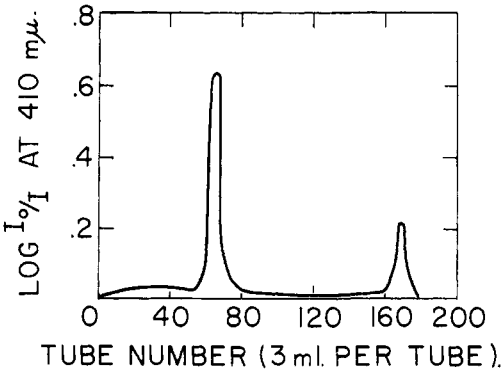

Fig. 1.

Paper chromatograms employing modifications of the solvent systems of Chu, *et al.*,[5] confirmed the results of column chromatography. Also each hemin fraction obtained from column chromatography resulted in a single fraction upon re-chromatographing on the silicic acid column. The porphyrins derived from the hemin fractions gave identical spectra typical of hematoporphyrin, with absorption bands at 404, 504, 536, 572 and 625 mμ. The spec-

(1) D. Keilin and E. F. Hartree, *Proc. Roy. Soc.* (*London*), **B122**, 298 (1937).
(2) E. Margoliash, *Biochem. J.*, **56**, 529 (1954).
(3) K. G. Paul, *Acta Chem. Scand.*, **5**, 389 (1951).
(4) M. Morrison and E. Stotz, *J. Biol. Chem.*, in press.
(5) T. C. Chu, A. A. Green and E. J. Chu, *ibid.*, **190**, 643 (1951).

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.

The authors wish to acknowledge the support of the Life Insurance Medical Fund and the National Heart Institute, National Institutes of Health for the work reported.

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

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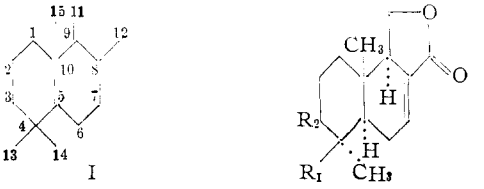
RECEIVED NOVEMBER 23, 1954

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

Sir:

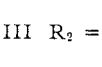
The fact that the bicyclofarnesol skeleton I,[2] present in all cyclic di- and tri-terpenes, has never been encountered among sesquiterpenes, has led to the suggestion by Ruzicka[3] in an outstanding review on the biogenesis of terpenes that "*this appears to indicate that the biogenesis of steroids, 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]



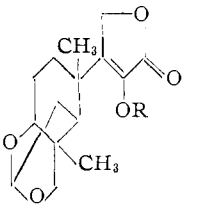
I

II R_2 =  ; $R_1 = CH_2OH$

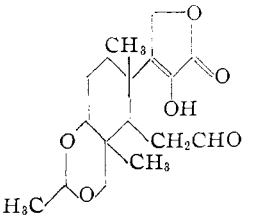
III R_2 =  ; $R_1 = CH_2OC(C_6H_5)_3$

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)).