pired as carbon dioxide since the activity of the nutrient solution was negligible. Although the total activity of the plants in the 21 day experiment was less than in the 7 day experiment, the activity of the crude alkaloid fraction had increased slightly.

It is considered that there was an initial rapid uptake of radioactive ornithine which was incorporated into proteins and a certain fraction into nicotine. Inactive ornithine was then synthesized by normal routes from inactive precursors. This inactive ornithine apparently exchanged with combined radioactive ornithine in proteins or other non-alkaloid plant components reducing the activity in the residual plant material. Some of the released radioactive ornithine would be incorporated into nicotine and some would be oxidized to carbon dioxide which on expiration would reduce the over-all activity in the plant. Since the nicotine fraction had a slightly higher activity after 21 days it appears that nicotine does not re-enter the general metabolic pool of the plant once it has been formed. Work is proceeding to see whether the nicotine remains indefinitely in the plant without undergoing metabolic change.

The activities of nicotine and its degradation products in the two experiments are almost identical and they can be discussed together. No activity was found in the N-methyl group. This result is in agreement with that previously obtained.[1a] Dewey, *et al.*,[2] detected a small amount of activity in the N-methyl group after feeding ornithine-2-C^{14} to tobacco plants. The plants used by these workers were younger than ours and possibly had not as large a photosynthetic capacity. Thus the plants were possibly deficient in carbon and would utilize some of the breakdown products of ornithine for the synthesis of N-methyl groups, albeit inefficiently. Our plants were grown in a strong draught, expired radioactive carbon dioxide being rapidly swept away. It has been reported[16] that excised tobacco leaves utilize carbon dioxide for the synthesis of the N-methyl group of nicotine.

The activity of the barium carbonate represents the activity of C-2 of nicotine. Since the activity of the nicotinic acid and its hydrochloride are almost identical with this, there must be negligible activity in the pyridine ring. Since no activity was found in the N-methyl group, the difference in the activity of the nicotine and the pyrazole (V) represents the activity of C-5. The difference in the activity of the pyrazole and nicotinic acid represents the activity of C-3 and C-4. This is negligible, thus all the activity in the nicotine is located on C-2 and C-5 and is equally divided between them. This confirms our previous hypothesis[1a] that ornithine is metabolized to a symmetrical four carbon compound before incorporation into the nicotine molecule.

Acknowledgment.—The authors are indebted to Dr. S. G. Wildman and Dr. A. Lang of the Department of Botany of this University for help in the cultivation of the tobacco plants.

(16) A. M. Kuzin and V. I. Merenova, *Doklady Akad. Nauk. SSSR.*, **85**, 393 (1952).

LOS ANGELES 24, CAL.

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE]

The Veratrum Alkaloids. XLI.[1] The Position of the Second Hydroxyl in Rubijervine and the Identity of Certain Dehydrogenation Products

BY S. WILLIAM PELLETIER AND DAVID M. LOCKE

RECEIVED MARCH 22, 1957

The second hydroxyl of rubijervine is shown to be at position 12 by infrared and optical rotatory dispersion studies on rubijervone-12. Reduction of this ketone with sodium-in-propanol gives 12-epirubijervine (IIIa), showing that the 12-hydroxyl in rubijervine (Ia) is axial. Molecular rotation data for rubijervine and 12-epirubijervine derivatives are also in agreement with assignment of the 12α- and 12β-configurations, respectively, to these compounds. The hydrocarbon $C_{18}H_{16}$ from the dehydrogenation of rubijervine is shown to be 1′-methyl-1,2-cyclopentenophenanthrene (VIII). The phenol $C_{18}H_{16}O$ obtained in the same dehydrogenation has been converted to 1′-methyl-1,2-cyclopentenophenanthrene (VIII) by reduction of its diethyl phosphate ester with sodium in liquid ammonia. Its infrared spectrum leads to the conclusion that it is 1′-methyl-1,2-cyclopentenophenanthrol-3 (X).

The various species of *Veratrum* elaborate a series of complex alkaloids, some of which have found use in combatting hypertensive disorders. The alkaloids fall into two broad classes.[2,3] The first includes a series of highly oxygenated alkamines of the formula $C_{27}H_{43}NO_{7-9}$, which occur in nature as esters of relatively simple organic acids. The second comprises a group of C_{27}-bases of low oxygen content which occur free or as glycosides. Rubijervine,[3,4] an example of the latter class, was first discovered by Wright and Luff[5] in *Veratrum album*[6] and has since been found in *Veratrum viride*.[7,8] Studies by Jacobs and Craig showed this alkaloid to have the molecular formula $C_{27}H_{43}NO_2$[9] and to be a hexacyclic tertiary steroidal base containing a 3β-hydroxy-Δ^5-stenol system and a second hydroxy

(1) A preliminary account of a portion of this work is outlined in Paper XL, D. M. Locke and S. W. Pelletier, *Chemistry & Industry*, 1049 (1956).

(2) V. Prelog and O. Jeger, in 'The Alkaloids, Chemistry and Physiology," edited by R. H. F. Manske and H. L. Holmes, Vol. III, Academic Press, Inc., New York, N. Y., 1953, p. 270.

(3) J. McKenna, *Quart. Revs.*, **7**, 231 (1953).

(4) Reference 2, pp. 277–280.

(5) C. R. A. Wright and A. P. Luff, *J. Chem. Soc.*, **35**, 405, 421 (1879).

(6) G. Salzberger, *Arch. Pharm.*, **228**, 462 (1890).

(7) E. J. Seiferle, I. B. Johns and C. H. Richardson, *J. Econ. Entomol.*, **35**, 35 (1942).

(8) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **160**, 555 (1945).

(9) W. A. Jacobs and L. C. Craig, *ibid.*, **148**, 41 (1943).

group.[10] Dehydrogenation of rubijervine with selenium afforded 2-ethyl-5-methylpyridine and a methylcyclopentenophenanthrene[9] and a phenanthrol, $C_{18}H_{16}O$,[9,11] both of uncertain identity. Rubijervine was subsequently shown to be a hydroxysolanidine by Sato and Jacobs who oxidized rubijervine monobenzoate to a ketone which was reduced by the Wolff–Kishner method to solanidine.[11] These workers also reduced diketodihydrorubijervine to solanidan-3β-ol. An assignment of the second hydroxyl group of rubijervine was made on the basis of the following argument. Since it is this hydroxyl which appears in the $C_{18}H_{16}O$ phenanthrol (no phenol was isolated from solanidine under the same conditions),[12] its position is thereby restricted to the A, B or C ring. The available data exclude all of these positions except carbon-12. Thus, rubijervine is stable to periodate; dihydrorubijervine gives a diketone which is stable to alkali, forms a disemicarbazone, fails to form a pyridazine with hydrazine and shows no interaction of the chromophores in the ultraviolet; rubijervone-12 shows no α,β-unsaturation in the ultraviolet.[11] In addition, the molecular rotation difference between diketodihydrorubijervine and solanidan-3-one is in agreement with that recorded for a 12-keto steroid.[13] The second hydroxyl in rubijervine was tentatively assigned the α-configuration by Sato and Jacobs[11] who noted that the molecular rotation difference between dihydrorubijervine and solanidan-3β-ol is comparable to that recorded for a 12α-hydroxy steroid.[13]

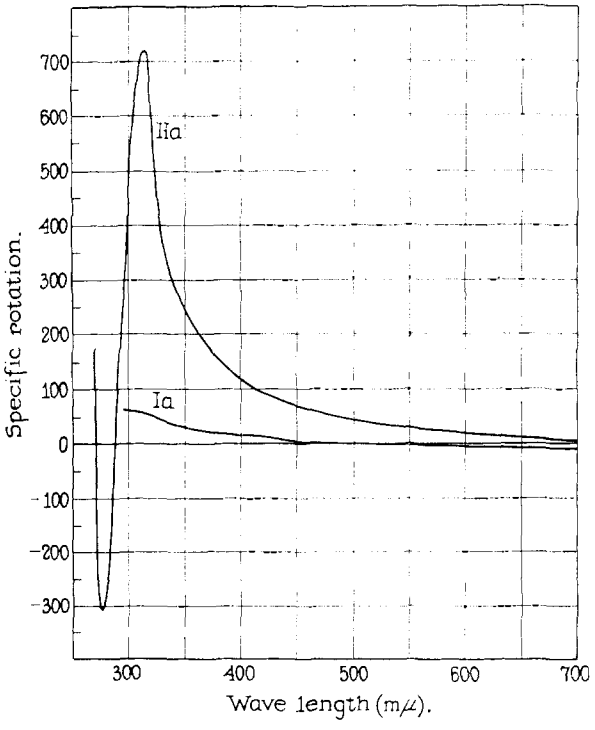


Fig. 1.—Rotatory dispersion curves[16] of: rubijervine (Ia) and rubijervone-12 (IIa) in dioxane.

Data have now been accumulated which demonstrate that rubijervine is indeed Δ^5-solanidene-3β,12α-diol. It is the purpose of this paper to present the details of this work and to discuss the identity of the hydrocarbon $C_{18}H_{16}$[9] and phenol $C_{18}H_{16}O$[9,11] obtained from the dehydrogenation of rubijervine.

The structure proposed requires that rubijervone-12 benzoate (IIb), produced by chromic acid oxidation of rubijervine 3-benzoate (Ib), should show absorption in the infrared for an unconjugated ketone in a six-membered ring. This compound shows, in fact, only single carbonyl absorption at 1708 cm.$^{-1}$, due to both the benzoyl and the keto groups.[14] Its hydrolysis product, rubijervone-12 (IIa), shows carbonyl absorption at 1706 cm.$^{-1}$.[15] More striking confirmation of the position of this carbonyl function is available from a comparison of the optical rotatory dispersion curves of rubijervine and rubijervone-12 (IIa) (Fig. 1),[16] which were determined in the laboratory of Professor Carl Djerassi. The curve of the ketone with a "maximum" of 720° at 313 mμ may be compared with those of a series of 12-keto bile acids which show "maxima" of 772–887° at 305–307 mμ[17] and with those of 12-ketosapogenins which show "maxima" of 424°, 783° and 802° at 312.5–315 mμ.[18] In the case of the bile acids the maxima are more positive than for rubijervone-12 because of the higher background rotation of the bile acid system. The curve is perfectly compatible with that expected for a 12-ketone, while an 11-ketone may be definitely excluded.

The configuration of the second hydroxyl in rubijervine was established by two lines of evidence, the first chemical and the second based on molecular rotation differences.

Thus, reduction of rubijervone-12 (IIa) by sodium-in-alcohol (a reaction which is known to be the equivalent of equilibration[19]) should in theory produce the epimer with a 12-equatorial hydroxyl,[20] *viz.*, Δ^5-solanidene-3β,12β-diol (IIIa). In practise, this reaction afforded in 79% yield, not rubijervine (see Fig. 2 for infrared spectra), but an isomer of m.p. 231–233°, $[\alpha]^{27}D$ −18°. The benzoate of the above ketone, Δ^5-solanidene-3β-ol-12-one benzoate (IIb), was similarly reduced to the same isomer IIIa. Catalytic reduction of IIIa over plat-

(10) W. A. Jacobs and L. C. Craig, (a) *J. Biol. Chem.*, **152**, 641 (1944); (b) **159**, 617 (1945).

(11) Y. Sato and W. A. Jacobs, *ibid.*, **179**, 623 (1949).

(12) L. C. Craig and W. A. Jacobs, *ibid.*, **149**, 451 (1943).

(13) D. H. R. Barton and W. Klyne, *Chemistry & Industry*, 755 (1948).

(14) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, pp. 114, 152.

(15) It should be noted that even if the position of the hydroxy is not restricted to the A, B or C ring, the 15-position is excluded by the infrared data. In addition, the 26-position is excluded since oxidation at this position should produce a lactam and not a ketone. It seems unlikely that a hydroxyl group would occur on the 23- or 24-position because of the isolation from the dehydrogenation mixture of 2-ethyl-5-methylpyridine and the failure to isolate a hydroxypyridine. Both jervine and veratramine contain oxygen functions at carbon-24 and give rise to hydroxypyridines on dehydrogenation. By this alternative argument, all positions in the D, E and F rings may be excluded as possible locations for the hydroxyl.

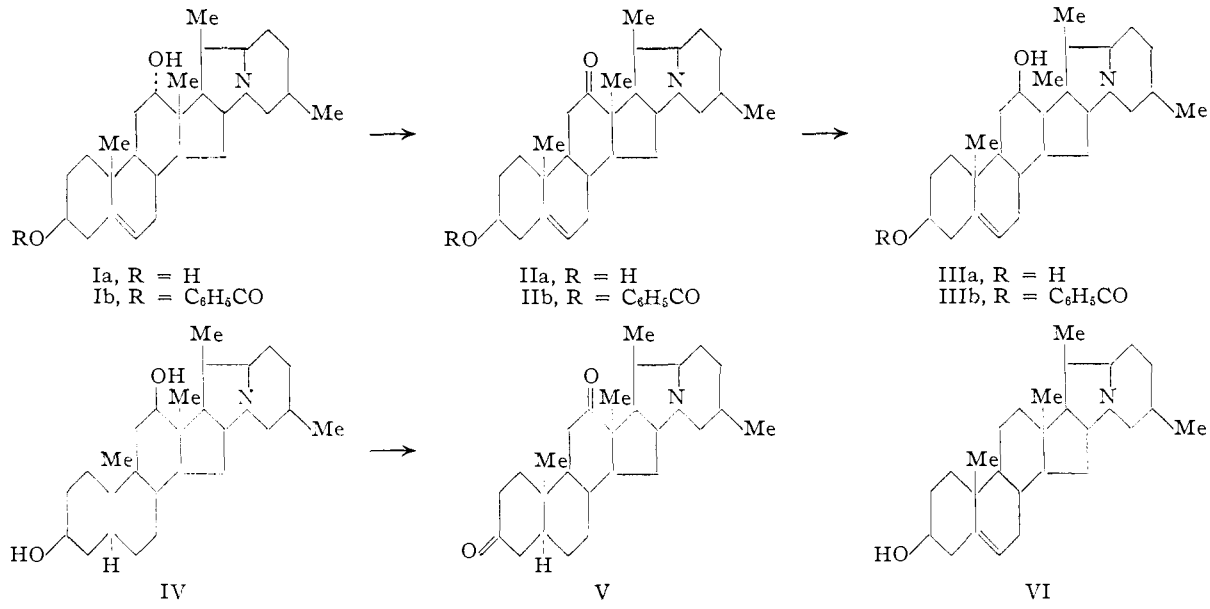
(16) We wish to express our grateful appreciation to Professor Carl Djerassi for supplying the rotatory dispersion curves presented in this paper and also for his advice in interpreting them.

(17) C. Djerassi and W. Closson, THIS JOURNAL, **78**, 3761 (1956).

(18) C. Djerassi and R. Ehrlich, *ibid.*, **78**, 440 (1956).

(19) M. G. Vavon, *Bull. soc. chim. France*, Ser. 4, **49**, 937 (1931); W. Hückel, *Ann.*, **533**, 1 (1937).

(20) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).



Ia, R = H
Ib, R = C_6H_5CO

IIa, R = H
IIb, R = C_6H_5CO

IIIa, R = H
IIIb, R = C_6H_5CO

IV V VI

inum oxide gave in 84% yield a dihydro compound, solanidane-3β-12β-diol (IV), m.p. 220–221°, $[\alpha]^{26}D$ 27°, different from dihydrorubijervine, but furnishing on oxidation with chromic acid the known diketodihydrorubijervine (V).[11] This series of reactions (together with those reported below) demonstrates that the product of sodium-in-alcohol is in fact 12-epirubijervine (IIIa) and possesses a hydroxyl with the 12β-configuration (equatorial).

lated in small yield rubijervine monobenzoate (Ib) and in slightly greater yield its 12-epimer, 12-epirubijervine 3-benzoate (IIIb), m.p. 230.5–231.5°, $[\alpha]^{26}D$ −4°. The structure of the latter was shown by its hydrolysis to 12-epirubijervine (IIIa).

It has been pointed out that the molecular rotation difference between dihydrorubijervine and solanidane-3β-ol is close to that recorded for a

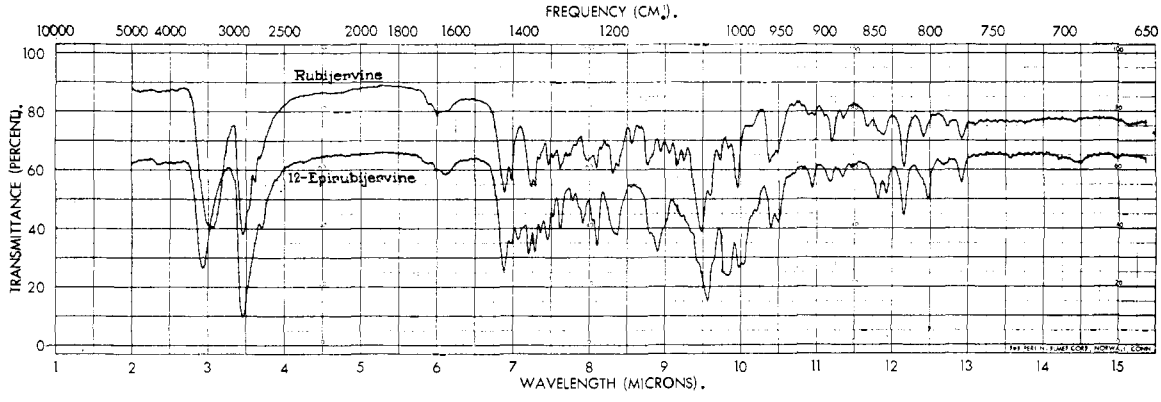


Fig. 2.—Infrared absorption spectra in KBr.

It is a general rule that lithium aluminum hydride or sodium borohydride reduces a highly hindered ketone to an axial hydroxyl and an unhindered ketone to an equatorial hydroxyl.[20,21] The 12-position in steroids is a moderately hindered one, and it is therefore not surprising that rockogenin, a 12-ketosapogenin, gives on lithium aluminum hydride reduction a mixture of epimeric 12-diols.[22] In agreement with these results, sodium borohydride reduction of rubijervone-12 benzoate (IIb) produced a mixture of epimers from which was iso-

(21) W. G. Dauben, G. J. Fonken and D. S. Noyce, THIS JOURNAL, **78**, 2579 (1956); W. G. Dauben, E. J. Blanz, Jr., J. Jiu and R. A. Micheli, *ibid.*, **78**, 3752 (1956); D. J. Cram and F. A. Abd Elhafez, *ibid.*, **74**, 5828 (1952).

(22) R. Hirschmann, C. S. Snoddy, Jr., C. F. Hiskey and N. L. Wendler, *ibid.*, **76**, 4013 (1954).

12α-hydroxy steroid. With a series of rubijervine derivatives which are epimeric at C-12 now available, the molecular rotation differences of both configurations may be compared with those of related compounds whose configuration at carbon-12 is known. In Table I are included a series of rubijervine and 12-epirubijervine derivatives together with appropriate steroids, sapogenins and bile acids. It will be noted that in a given epimeric pair, the molecular rotation difference for the 12α-epimer is always more strongly positive than for the 12β-isomer. Thus the molecular rotation differences support the assignment of the 12α-configuration to compounds of the rubijervine series and the 12β configuration to those of the 12-epirubijervine series.

TABLE I

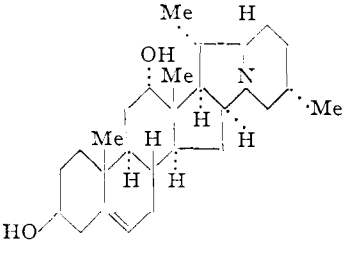
MOLECULAR ROTATION CONTRIBUTIONS OF 12α- AND 12β-HYDROXYL GROUPS

$\Delta OH = [M]_D CHOH - [M]_D CH_2$

Compound	$[M]_D$. CHOH	$[M]_D CH_2$	$\Delta OH_{12\alpha}$	$\Delta OH_{12\beta}$
Rubijervine (Ia)	33[a]	−107[b]	140	
12-Epirubijervine (IIIa)	− 74[a]	−107[b]		33
Dihydrorubijervine	220[c]	113[b]	107	
Solanidane-3β,12β-diol (IV)	112[a]	113[b]		− 1
Rubijervine monobenzoate (Ib)	114[a]	− 30[a]	144	
12-Epirubijervine 3-benzoate (IIIb)	− 21[a]	− 30[a]		9
12-Epirockogenin	−182[d]	−204[e]	22	
Rockogenin	−292[d]	−204[e]		−88
12α-Hydroxy steroid[f]	....	....	93	
12β-Hydroxy steroid[f]	....	....		50
Methyl 3α,12α-dihydroxyetianate	283[g]	154[h]	129	
Methyl 3α-12β-dihydroxyetianate	60[g]	154[h]		−94
3α,12α-Dihydroxycholanic acid	208[i]	120[i]	88	
3α,12β-Dihydroxycholanic acid	149[i]	120[i]		29

[a] Reported in this paper. [b] V. Prelog and S. Szpilfogel, *Helv. Chim. Acta*, **27**, 390 (1944). [c] Y. Sato and W. A. Jacobs, *J. Biol. Chem.*, **179**, 623 (1949). [d] R. Hirschmann, C. S. Snoddy, Jr., C. F. Hiskey and N. L. Wendler, THIS JOURNAL, **76**, 4013 (1954). [e] L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 3rd ed., 1949, p. 591. [f] D. H. R. Barton and W. Klyne, *Chemistry & Industry*, 755 (1948). [g] S. Pataki, K. Meyer and T. Reichstein, *Helv. Chim. Acta*, **36**, 1295 (1953). [h] Value for etiolithocholic acid, T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, **23**, 658 (1940). [i] L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publishing Corp., New York, N. Y., 1949, p. 108.

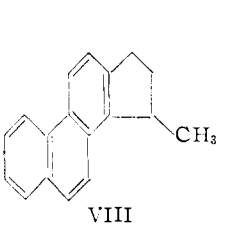
The complete stereochemistry of rubijervine, based on that derived for solanidine,[23] is indicated in formula VII. Although some uncertainty still exists about the configuration at several centers in the E and F rings, particularly at C-22, the proposed solanidine structure is compatible with the known stereochemical data.



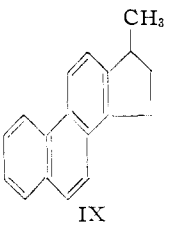
VII, rubijervine

Since the configurations of the hydroxyls in rubijervine and its derivatives now appear well established, it is interesting to consider the assignment of infrared absorption bands in the C–O stretching region to the different types of hydroxyls. The appropriate bands are given in Table II. Solanidine, epirubijervine and rubijervine contain the Δ^5-3β-hydroxy system which appears to be responsible for the absorption at 1048–1047 and 1018–1016 cm.$^{-1}$ [24] (Δ^5-3β-hydroxy steroids, 1052–1050 cm.$^{-1}$).[25] In dihydrorubijervine the 3β-hydroxy allo system absorbs at 1037 cm.$^{-1}$ (3β-hydroxy allo steroids, 1040–1037 cm.$^{-1}$).[25,26] Epirubijervine contains, in addition to the Δ^5-3β-hydroxy system, a 12β (equatorial) hydroxyl which apparently absorbs at 1052 cm.$^{-1}$, although its absorption may coincide with that of the equatorial hydroxyl at C-3. The absorption of rubijervine and dihydrorubijervine at 1028–1026 cm.$^{-1}$ may be assigned to the 12α (axial) hydroxyl. If these assignments are correct, the 12-equatorial hydroxyl absorbs at a higher frequency than the 12-axial epimer. This situation parallels that found for the 2-, 3- and 4-positions in the steroids,[25,27] but the reverse is true for the 3-position in the triterpenes.[28]

In the course of a study of the dehydrogenation products of rubijervine, Jacobs and Craig isolated a hydrocarbon $C_{18}H_{16}$ having an ultraviolet absorption spectrum similar to that of cyclopentenophenanthrene.[9] Its melting point and that of its TNB adduct were very close to the corresponding constants reported for 1′-methyl-1,2-cyclopentenophenanthrene.[29] It is difficult to rationalize the formation from rubijervine of a hydrocarbon of



VIII



IX

such a structure. Solanidine under similar conditions furnishes Diels' hydrocarbon (IX)[12,30] as well as 2-methyl- and 1,2-dimethylphenanthrene.[12] As with most steroids,[31,32] Diels' hydrocarbon presumably results from migration of the C-18 methyl to the C-17 position accompanying cleavage of the side chain. Cyclopentenophenanthrene is obtained from the dehydrogenation of isorubijervine, Δ^5-

(23) (a) Y. Sato and H. G. Latham, Jr., *Chemistry & Industry*, 444 (1955); (b) THIS JOURNAL, **78**, 3146 (1956).

(24) The band at 1001–1004 occurs in all samples and is apparently not of diagnostic significance. The band occurring at 995–997 in epirubijervine and dihydorubijervine cannot be assigned readily.

(25) A. R. H. Cole, R. N. Jones and K. Dobriner, THIS JOURNAL, **74**, 5571 (1952); W. G. Dauben, E. Hoerger and N. K. Freeman, *ibid.*, **74**, 5206 (1952).

(26) H. Rosenkrantz and L. Zablow, *ibid.*, **75**, 903 (1953).

(27) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, *ibid.*, **73**, 3215 (1951); A. Fürst, H. H. Kuhn, R. Scotoni, Jr., and Hs. H. Günthard, *Helv. Chim. Acta*, **35**, 951 (1952); A. R. H. Cole, *J. Chem. Soc.*, 4969 (1952).

(28) I. L. Allsop, A. R. H. Cole, D. E. White and R. L. S. Willix, *ibid.*, 4868 (1956).

(29) L. Ruzicka, L. Ehmann, M. W. Goldberg and H. Hösli, *Helv. Chim. Acta*, **16**, 833 (1933).

(30) H. Rochelmeyer, *Arch. Pharm.*, **274**, 543 (1936); A. Soltys and K. Wallenfels, *Ber.*, **69**, 811 (1936).

(31) P. A. Plattner, "Newer Methods of Preparative Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1948, p. 48.

(32) J. W. Cook, *Chemistry & Industry*, **13**, 176 (1935); E. Bergmann, *ibid.*, **13**, 175 (1935); O. Rosenheim and H. King, *ibid.*, **11**, 299 (1933); A. Cohen, J. W. Cook and C. L. Hewett, *J. Chem. Soc.* 445 (1935).

TABLE II

POSITION OF THE C–OH STRETCHING BANDS OF RUBIJERVINE AND RELATED COMPOUNDS

Compound	Substitution type	Frequency,[a] cm.$^{-1}$					
Solanidine (VI)	Δ^5,3β-OH(e)		1047s		1018m	1003w	
Epirubijervine (IIIa)	Δ^5,3β-OH(e); 12β-OH(e)	1052m	1047s		1018m	1004m	997m
Rubijervine (Ia)	Δ^5,3β-OH(e); 12α-OH(a)		1048s	1028m	1016m	1003m	
Dihydrorubijervine	5α-H,3β-OH(e); 12α-OH(a)		1037s	1026m		1001m	995m

[a] 5% solutions in CS_2 in 2-mm. cell; s = strong, m = medium, w = weak.

solanidene-3β,18-diol.[10b] In this case, it is presumed that the C_{18}-hydroxyl interferes with the expected migration of this group to the 17-position during the dehydrogenation. However, with rubijervine there seems to be no simple explanation for the presumed appearance of a methyl group at the 1′-position of cyclopentenophenanthrene. Several different structural features may lead to methyl migrations during dehydrogenation. For example, in addition to the methyl group migration in the production of Diels' hydrocarbon there are instances where a methyl migrates to an unsubstituted position on the aromatic nucleus.[33] Cases are also known in which a methyl group migrates to a position occupied by a hydroxyl, presumably by a retropinacol rearrangement, prior to the dehydrogenation proper.[34] The unique feature of the dehydrogenation of rubijervine is the apparent methyl migration to an unsubstituted methylene on the non-aromatic portion of the molecule, two carbon atoms removed from its original site.

These considerations seemed to warrant a further study of the identity of this hydrocarbon. Accordingly, a sample of the hydrocarbon from the original dehydrogenation,[9] which was placed at our disposal by Drs. Jacobs and Craig, was purified by repeated chromatography and crystallization to give material melting at 78–80° cor. and with an ultraviolet absorption spectrum almost identical with Diels' hydrocarbon[35] IX and with cyclopentenophenanthrene.[36] A new analysis confirmed its formula as $C_{18}H_{16}$. Synthetic 1′-methyl-1,2-cyclopentenophenanthrene[29] was unavailable for direct comparison, but 3-methyl-1,2-cyclopentenophenanthrene,[37,38] m.p. 86–87°, and 5-methyl-1,2-cyclopentenophenanthrene,[38,39] m.p. 75–76°, both showed melting point depressions with the hydrocarbon from rubijervine. The infrared spectrum of the hydrocarbon from rubijervine is included in Fig. 3. It is appreciably different from all of the recorded spectra[40] for the methyl-1,2-cyclopentenophenanthrenes, which include all but the 1′-, 2′- and 7-isomers. Since the melting points[29,41] of the last two isomers and/or of their derivatives are different from those of the hydrocarbon from rubijervine, only 1′-methylcyclopentenophenanthrene remained as a possibility. Accordingly, the synthesis of this compound by the method of Ruzicka, *et al.*,[29] was repeated (with minor modification)[42] to give material of m.p. 79–81°, mixture m.p. with the hydrocarbon from rubijervine, 78–80°. The ultraviolet and infrared absorption spectra (Fig. 3) of the latter were identical with those of the synthetic sample. In addition, the picrates and trinitrobenzene adducts of each hydrocarbon showed no melting point depression on admixture. Thus, the identity of the $C_{18}H_{16}$ hydrocarbon from rubijervine with 1′-methyl-

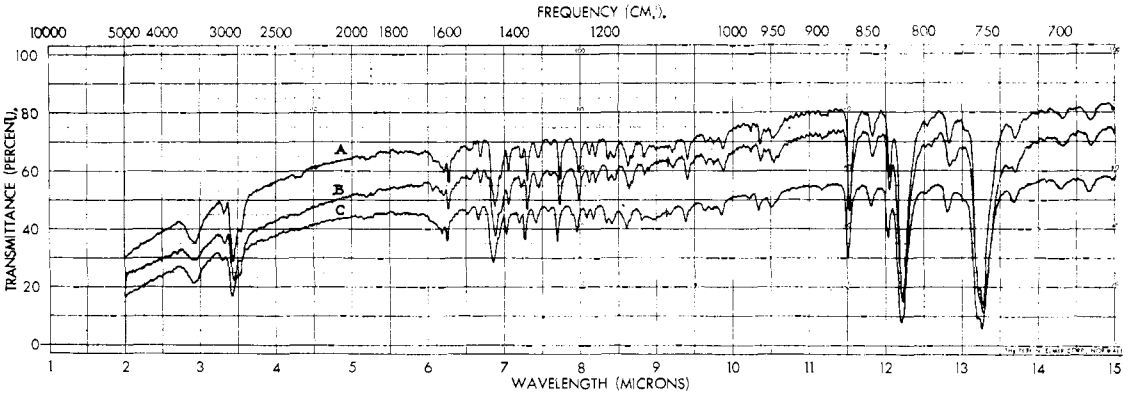


Fig. 3.—Infrared absorption spectra of 1′-methyl-1,2-cyclopentenophenanthrene in KBr: A, from dehydrogenation of rubijervine; B, from reduction of $C_{18}H_{18}O$ phenol; C, synthetic.

(33) R. D. Haworth, C. R. Mavin and G. Sheldrick, *J. Chem. Soc.*, 454 (1934).

(34) L. Ruzicka, M. W. Goldberg and K. Hofmann, *Helv. Chim. Acta*, **20**, 325 (1937); L. Ruzicka, H. Schellenberg and M. W. Goldberg, *ibid.*, **20**, 791 (1937).

(35) S. H. Harper, G. A. R. Kon and F. C. J. Ruzicka, *J. Chem. Soc.*, 124 (1934).

(36) W. V. Mayneord and E. M. F. Roe, *Proc. Roy. Soc. (London)*, Ser. A, **152**, 299 (1935).

(37) A. Butenandt, H. Dannenberg and D. von Dresler, *Z. Naturforsch.*, **1**, 151 (1946); B. Riegel, S. Siegel and D. Kritchevsky, THIS JOURNAL, **70**, 2950 (1948).

(38) We wish to thank Professor Butenandt for samples of 3-methyl- and 5-methylcyclopentenophenanthrene and Dr. Riegel for a sample of 3-methyl-cyclopentenophenanthrene and its TNB and TNF derivatives.

(39) A. Butenandt, H. Dannenberg, E. Bieneck and W. Steidle, *Z. Naturforsch.*, **5b**, 405 (1950).

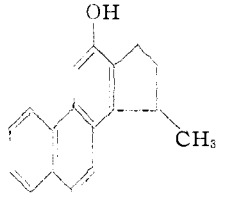
(40) H. Dannenberg, U. Schiedt and W. Steidle, *ibid.*, **8b**, 269 (1953).

(41) A. Butenandt, H. Dannenberg and D. von Dresler, *ibid.*, **1**, 227 (1946).

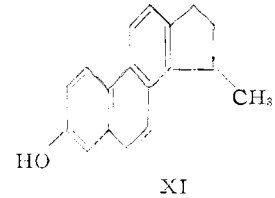
(42) The required 2-(1-naphthyl)-ethanol was prepared in 76% yield by reduction of 1-naphthylacetic acid with lithium aluminum hydride.

1,2-cyclopentenophenanthrene is established.

A related question concerns the formation of the phenanthrol $C_{18}H_{16}O$, m.p. 136–138°, obtained in the same dehydrogenation.[9] In order to provide more information concerning this phenol, which was kindly supplied by Drs. Jacobs and Craig, several attempts were made to convert it to the corresponding hydrocarbon by heating with zinc dust. At temperatures high enough to effect reduction, rearrangement occurred since only a high melting hydrocarbon fraction could be obtained which had the ultraviolet spectrum of chrysene. Recently Kenner and Williams[43] have reported a mild method for the conversion of phenols to aromatic hydrocarbons, which involves reduction of the aryl diethyl phosphate with sodium in liquid ammonia. Since the method had been tested only with benzene and naphthalene derivatives, its application to phenols of higher molecular weight was first explored by reduction of 3-phenanthrol,[44] chosen as a suitable model for the phenol from rubijervine. The reduction proceeded smoothly to give phenanthrene in 30% yield. Reduction of the phenol from rubijervine by this method gave in 36% yield, 1′-methyl-1,2-cyclopentenophenanthrene, identified by mixture melting point, infrared (Fig. 3) and ultraviolet absorption spectra. The hydroxyl of this phenol has been assumed (*vide supra*) to be derived from the second hydroxyl (position-12) of rubijervine. If so, the phenol can be assigned the structure 1′-methyl-1,2-cyclopentenophenanthrol-3 (X). If, however, the hydroxyl is derived from the 3-hydroxyl of rubijervine, the structure should be 1′-methyl-1,2-cyclopentenophenanthrol-7 (XI). The infrared spectrum of the $C_{18}H_{16}O$ phenol provides a clear choice between these alternatives. A study of the frequencies associated with the aromatic C–H deformations in a series of fifteen cyclopentenophenanthrenes shows



X



XI

that without exception compounds having four adjacent ring hydrogens (*i.e.*, an unsubstituted A-ring) show strong absorption at 741–762 cm.$^{-1}$ [40]. The only other situation which causes strong absorption in this region is the presence of three adjacent ring hydrogens,[45] and these occur in neither X nor XI. Since the phenol from rubijervine shows strong absorption at 748 cm.$^{-1}$ [46] it appears that the A-ring is unsubstituted and that the phenol therefore has structure X. Regardless of the precise structure of the phenol, the fact that it is a derivative of 1′-methylcyclopentenophenanthrene demonstrates that the methyl group is able to migrate without prior, or simultaneous, loss of the hydroxyl from the molecule. On the other hand, the hydroxyl seems to be implicated in this migration in some fashion since solanidine (VI), in which this hydroxyl is absent, gives Diels' hydrocarbon. Thus the precise mode of formation of the dehydrogenation products from rubijervine remains to be explained.

Acknowledgment.—We wish to express our appreciation to The Lilly Research Laboratories, Eli Lilly and Co., for a generous grant in support of this work. We also wish to thank Dr. Walter A. Jacobs who kindly read the manuscript and who placed his collection of samples at our disposal. Analyses are by Mr. D. Rigakos of this Laboratory. The technical assistance of Miss Vera Bohan is gratefully acknowledged.

Experimental

General Experimental Procedures.—Boiling points are uncorrected. Melting points are corrected and were taken on a hot-stage equipped with a polarizer. Finely powdered samples were placed on the stage about 15° below the melting point and the temperature raised rapidly to within 5° of the melting point. The temperature was then raised 2°/min.

Petroleum ether refers to a light petroleum fraction of b.p. 30–60°. Ligroin refers to a light petroleum fraction of b.p. 60–70°.

The removal of solvents *in vacuo* was accomplished with a rotating flash evaporator at about 15 mm. and with a heating bath operated usually at about 35–40°.

Infrared spectra were determined from 2 to 15 μ without compensation on a Perkin–Elmer model 21 double beam spectrometer with sodium chloride optics, set at resolution 927, response 2, gain 6, suppression 2 and a scanning speed of 0.3 μ/minute on a chart scale of 5 cm. for 1 μ. Ultraviolet spectra were determined in 95% ethanol on a Beckman model DU spectrophotometer.

Rubijervine.—The sample of rubijervine[9,47] used for the transformations reported in this work was purified by recrystallization from 95% ethyl alcohol. It consisted of colorless rods, m.p. 242–244°, $[\alpha]^{27.5}D$ +8° (*c* 0.54, chf.) $[\alpha]^{27.5}D$ +20° (*c* 1.04, MeOH), $[\alpha]^{27.5}D$ +11 (*c* 1.00, dioxane).[48]

Anal. Calcd. for $C_{27}H_{43}O_2N$: C, 78.40; H, 10.48. Found: C, 78.08; H, 10.23.

Solanidine Benzoate.—A sample of solanidine benzoate,[49] prepared by benzoylation of solanidine in pyridine, had m.p. 213–214°, $[\alpha]^{27}D$ −6° (*c* 1.40, chf.).

Anal. Calcd. for $C_{34}H_{47}O_2N$: C, 81.39; H, 9.44. Found: C, 81.44; H, 9.27.

Rubijervine 3-Benzoate.—The monobenzoate of rubijervine was prepared as reported by Sato and Jacobs[11] and was isolated directly from the reaction mixture by crystallization from benzene without recourse to chromatography. Colorless prisms were obtained, m.p. 256–259°, $[\alpha]^{28}D$ +22° (*c* 1.60, chf.).

Anal. Calcd. for $C_{34}H_{47}O_3N$: C, 78.87; H, 9.15. Found: C, 78.94; H, 8.97.

Rubijervone-12 Benzoate.—Oxidation of rubijervine monobenzoate according to the directions of Sato and Jacobs[11] gave rubijervone-12 benzoate, m.p. 227–231°, with a prior change in crystalline form at *ca.* 210°. A double melting point is reported, 214–216°, 233–234°. This compound showed in the infrared (Nujol) a single peak in the carbonyl region at 1708 cm.$^{-1}$.

(43) G. W. Kenner and N. R. Williams, *J. Chem. Soc.*, 522 (1955).

(44) We wish to thank Professor L. F. Fieser for a generous sample of 3-phenanthrol.

(45) This type of substitution is characterized by an additional absorption band at 786–792. The phenol from rubijervine shows no absorption in this region.

(46) *Cf.* the spectrum of 1,-methylcyclopentenophenanthrene which has a strong band at 754 cm.$^{-1}$ (KBr) Fig. 3.

(47) The authors wish to thank Dr. A. Stoll of Sandoz for placing at their disposal a generous sample of rubijervine.

(48) *Cf.* Fig. 1. The values obtained for $[\alpha]D$ (dioxane) in Prof. Djerassi's rotatory dispersion studies were −4° (*c* 0.05) and −7° (*c* 0.095). The accuracy at the sodium D-line is rather poor, due to the dilute solutions used.

(49) F. Bergel and R. Wagner, *Ber.*, **66**, 1093 (1933).

Rubijervone-12.—Hydrolysis of rubijervone-12 benzoate gave material melting at 229.5–232°. In our hands the sample prepared by Sato and Jacobs[11] melted at 226–228° (reported[11] m.p. 236–238° (uncor.)). A mixture of these two samples showed intermediate melting. The infrared spectrum (Nujol) of this compound shows hydroxyl absorption at 3185 cm.$^{-1}$ and carbonyl absorption at 1706 cm.$^{-1}$.

12-Epirubijervine. A. From Rubijervone-12.—A solution of 352 mg. of rubijervone-12 in 20 ml. of refluxing *n*-propyl alcohol was saturated slowly with small pieces of sodium over a period of several hours. After cooling to room temperature, the mixture was treated with several drops of ethyl alcohol and evaporated to dryness *in vacuo*. The residue was taken up in benzene and water. The aqueous layer was extracted with benzene, and the combined benzene extracts were washed with a small amount of water, which gave rise to an emulsion. Filtration of the mixture gave a crystalline solid, m.p. 226–228°. The benzene layer was evaporated to dryness *in vacuo* giving further crystalline material melting at 224–228°, showing partial melting at 208°. The former gave on recrystallization from benzene 123 mg. of large rods converted above 160° to tiny needles, m.p. 230–231°. The latter gave 155 mg. of material showing the same prior change in crystalline form and a final melting point of 224–228°.

An analytical sample was prepared by two recrystallizations from acetone, m.p. 231–233°, $[\alpha]^{27}D$ −18° (*c* 1.07, chf.). The infrared absorption spectrum is included in Fig. 2.

Anal. Calcd. for $C_{27}H_{43}O_2N$: C, 78.40; H, 10.48. Found: C, 78.46; H, 10.56.

B. From Rubijervone-12 Benzoate.—A solution of 90 mg. of rubijervone-12 benzoate in 5 ml. of refluxing *n*-propyl alcohol was saturated with sodium and worked up as above (without emulsion formation) to give 87 mg. of crude product, m.p. 226–229°, with the same preliminary change in crystalline form as noted above. Recrystallization from acetone gave needles, m.p. 232–234°. A mixture of this sample with an authentic sample of rubijervine (m.p. 240–242°) melted at 222–229°.

Solanidane-3β,12β-diol.—A solution of 55 mg. of 12-epirubijervine in methanol containing a small amount of acetic acid was treated with hydrogen in the presence of 28 mg. of platinum oxide. In 1 hr. one mole of hydrogen was consumed. The catalyst was then removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. The residue was recrystallized from ether to give 46 mg. of needles, m.p. 218–220°. An analytical sample prepared by several recrystallizations from acetone–ligroin melted at 220–221°, $[\alpha]^{26}D$ +27 (*c* 1.45, chf.).

Anal. Calcd. for $C_{27}H_{45}O_2N$: C, 78.02; H, 10.91. Found: C, 78.13; H, 11.17.

Solanidane-3,12-dione.—To a solution of 12 mg. of solanidane-3β,12β-diol in 2 ml. of glacial acetic acid was added 5 mg. of chromic oxide in 1 ml. of 90% acetic acid. After standing at room temperature for 1 hr., the solution was poured into 25 ml. of water and extracted with chloroform. The chloroform extracts were washed successively with sodium bicarbonate and water, dried and evaporated to dryness *in vacuo* to give 5 mg. of plates, m.p. 237–239°, undepressed on admixture with an authentic sample.[11]

Sodium Borohydride Reduction of Rubijervone-12 Benzoate. 12-Epirubijervine 3-Benzoate.—To a solution of 54 mg. of rubijervone-12 benzoate in 50 cc. of ether–methanol (60/40) was added 167 mg. of sodium borohydride. After standing at room temperature for 3 hr., the solution was evaporated to dryness *in vacuo*. The residue was taken up in chloroform and water, and the aqueous layer was extracted with chloroform. Concentration of the chloroform extracts gave 54 mg. of crude material, m.p. 215–230° (softens *ca.* 205°).

A partial fractional crystallization of this material furnished two fractions, one of m.p. 252–254° and the other with m.p. 220–222°. From the former was isolated by chromatography over alumina and recrystallization from benzene 4 mg. of crystals, m.p. 255–259°, undepressed on admixture with an authentic sample of rubijervine benzoate.

The 220–222° fraction on chromatography over alumina and subsequent recrystallization from ligroin furnished 16.5 mg. of tiny, fine needles of 12-epirubijervine 3-benzoate, m.p. 230.5–231.5°, $[\alpha]^{26}D$ −4° (*c* 1.53, chf.).

Anal. Calcd. for $C_{34}H_{45}O_3N$: C, 79.18; H, 8.80. Found: C, 79.10; H, 9.07.

Hydrolysis of 12-Epirubijervine 3-Benzoate.—A solution of 5 mg. of 12-epirubijervine 3-benzoate in 3 ml. of 1% methanolic potassium hydroxide was refluxed on the steam-bath for 0.5 hr. The solution was then evaporated to dryness *in vacuo* and taken up in chloroform and water. The chloroform layer was washed with water, dried and evaporated to dryness *in vacuo*. The residue was crystallized from acetone to give 3 mg. of long rods, m.p. 234–236°. A mixture with 12-epirubijervine (m.p. 231–233°) melted at 232–235°.

1'-Methyl-1,2-cyclopentenophenanthrene. A. From Rubijervine.—The original sample of the hydrocarbon obtained by Jacobs and Craig[9] from the selenium dehydrogenation of rubijervine was subjected to repeated chromatography over alumina and recrystallization from petroleum ether at 5°. This procedure gave material melting at 78–80° with an ultraviolet absorption spectrum almost identical with Diels' hydrocarbon[35] and with cyclopentenophenanthrene.[36] Its infrared spectrum showed aromatic C–H bands at 868, 830, 819, 758 sh. and 754 cm.$^{-1}$ (Fig. 3).

Anal. Calcd. for $C_{18}H_{16}$: C, 93.06; H, 6.94. Found: C, 92.88; H, 6.80.

The melting point of this material was strongly depressed on admixture with samples of 3-methyl-1,2-cyclopentenophenanthrene[37,38] and 5-methyl-1,2-cyclopentenophenanthrene.[38,39] It was not depressed on admixture with 1'-methyl-1,2-cyclopentenophenanthrene as prepared below. The infrared (Fig. 3) and ultraviolet spectra of the hydrocarbon from rubijervine were identical with those of 1'-methyl-1,2-cyclopentenophenanthrene.

A picrate of this hydrocarbon prepared in methanol melted at 130.5–131.5° (reported 131–132°).[9] A mixture melting point with a sample of the picrate from synthetic 1'-methyl-1,2-cyclopentenophenanthrene (described below) was not depressed.

A TNB of the hydrocarbon from rubijervine prepared in methanol melted at 143–143.5° (reported[9] 144–145°). A mixture melting point with authentic 1'-methyl-1,2-cyclopentenophenanthrene TNB was not depressed.

B. **Synthetic.**—The synthesis of **2-(1-naphthyl)-ethanol** was accomplished by lithium aluminum hydride reduction of 1-naphthylacetic acid. Reduction of 25 g. of acid for 1 hr. in boiling ether with 5.1 g. of lithium aluminum hydride gave 17.5 g. of 2-(1-naphthyl)-ethanol, m.p. 59–61° (reported 62°).[50] This alcohol was converted to **2-(1-naphthyl)-ethyl bromide**, b.p. 130–135° at *ca.* 0.3 mm., reported[51] b.p. 145–148° at 0.3 mm., in 88% yield by the method of Haworth and Mavin.[51] **Ethyl 5-methylcyclopentanone-2-carboxylate** was synthesized according to the directions of Cornubert and Borrel[52] giving material boiling at 114–119° at *ca.* 14 mm., reported[53] b.p. 105° at 12 mm. The condensation of β-(1-naphthyl)-ethyl bromide with ethyl 5-methylcyclopentanone-2-carboxylate was carried out by the directions of Kon[54] to give the β-keto ester, b.p. 195–208° at *ca.* 1 mm. (reported[54] b.p. 227° at 4 mm.) in 56% yield. This material (8.3 g.) was cyclized and aromatized by the method of Ruzicka, Ehmann, Goldberg and Hösli.[29] The product was isolated from the reaction mixture by chromatography over alumina. Elution with light petroleum gave a first fraction of 2.5 g. of a colorless oil with a blue fluorescence, which partially crystallized on standing. Further elution with the same solvent gave 2.0 g. of faintly yellow oil with a blue fluorescence, which crystallized completely on standing, m.p. 72–76°. Recrystallization of this latter material from ethanol gave 1.5 g. of material of m.p. 78–80°. Further recrystallization raised the melting point to 79–81° (reported[29] m.p. 76–77°).

A picrate of this hydrocarbon was prepared in methanol, m.p. 131–132°. *Anal.* Calcd. for $C_{18}H_{16}C_6H_3N_3O_7$: C, 62.47; H, 4.15. Found: C, 62.78; H, 4.04.

The TNB derivative prepared in methanol melted at 143–144° (reported 143–144°). *Anal.* Calcd. for $C_{18}H_{16}$-$C_6H_3N_3O_6$: C, 64.71; H, 4.30. Found: C, 64.86; H, 4.23.

(50) V. Grignard, *Compt. rend.*, **141**, 44 (1905).

(51) R. D. Haworth and C. R. Mavin, *J. Chem. Soc.*, 1012 (1933).

(52) R. Cornubert and C. Borrel, *Bull. soc. chim. France*, Ser. 4, **47**, 301 (1930).

(53) L. Bouveault and R. Locquin, *Compt. rend.*, **146**, 138 (1908).

(54) G. A. R. Kon, *J. Chem. Soc.*, 1081 (1933).

The Phenol $C_{18}H_{16}O$ from Dehydrogenation of Rubijervine.—A sample of the material obtained by Jacobs and Craig[9] was used without further purification for the experiments reported in this paper. In our hands it melted at 130–132° (reported[9] m.p. 136–138°). Its infrared spectrum (KBr) was characterized by bands at 3240 cm.$^{-1}$ (OH stretching) and 859, 853, 844, 827, 817, 755sh. and 748 cm.$^{-1}$ (aromatic C–H).

Reduction of the Phenol $C_{18}H_{16}O$ with Zinc Dust.—A mixture of 55 mg. of the phenol and 2.1 g. of zinc dust was heated in a sealed tube at 350–360° for 2 hr. After cooling to room temperature, the tube was opened and the contents were extracted repeatedly with benzene. On evaporation to dryness *in vacuo*, the benzene solution gave 39 mg. of a dark brown gum. This material was chromatographed over alumina giving 8 mg. of brown oil on elution with benzene and an additional 30 mg. of brown oil on elution with ether and methanol. The former resisted attempts at further purification and the latter furnished, on rechromatography and recrystallization (Norit) from ether, colorless needles, m.p. 133–134°, undepressed on admixture with starting material.

A mixture of 51 mg. of the phenol and 1.0 g. of zinc dust was heated at 400 ± 10° for 6 hr. to give 18 mg. of crude product which on chromatography over alumina gave on elution with benzene 11.5 mg. of an amorphous colorless glass. This material was crystallized from ethanol to give irregular crystals subliming above 220°, with a melting point *ca.* 242–248°, much dependent on the rate of heating. The melting point of a mixture of this material and a sample of chrysene (m.p. 256–258°) was 252–256°. The ultraviolet absorption spectrum was identical with that reported for chrysene[55] within experimental error.

Reduction of 3-Phenanthrol to Phenanthrene *via* Its Diethyl Phosphate Ester.[43]—Solutions containing 0.28 mmole of diethyl phosphite[56] and of triethylamine in carbon tetrachloride were prepared. A 52-mg. (0.26 mmole) sample of 3-phenanthrol[44] was dissolved in a 1-ml. aliquot of the diethyl phosphite solution, and this solution was cooled in an ice-bath. A 1-ml. aliquot of the triethylamine solution was then added, and the solution was allowed to stand at room temperature overnight. An additional 10 ml. of carbon tetrachloride was added, and the solution was washed with dilute hydrochloric acid and cold dilute sodium hydroxide (four times) and finally water. The organic layer was evaporated to dryness *in vacuo* giving 67 mg. of light brown oil. This material was taken up in a minimum of ether and cooled in a Dry Ice–Cellosolve-bath. A large excess of liquid ammonia was then run in until only one liquid phase was present and a solid had separated. The solution was then removed from the bath, and when it had reached the boiling temperature of the liquid ammonia several drops of ether were added to bring the solid into solution. Small pieces of sodium were added until a permanent blue color developed. Several drops of alcohol were added, and the solution was allowed to evaporate overnight. The residue was taken up in ether, washed with sodium bicarbonate, sodium hydroxide and finally water and then dried and evaporated to dryness *in vacuo* to give 27 mg. of nearly colorless oil. This was chromatographed over alumina to give 14.5 mg. of white crystals, m.p. 83–91°. Recrystallization from ethanol gave colorless scales 95–98°, undepressed when mixed with authentic phenanthrene. The infrared absorption spectrum of this material in KBr was identical with that of an authentic sample.

Reduction of the Phenol $C_{18}H_{16}O$ *via* Its Diethyl Phosphate Ester.—A sample of 45 mg. of the phenol from the dehydrogenation of rubijervine was converted to its diethyl phosphate ester (54 mg.) by the above procedure. Reduction was accomplished as above, 11 mg. (2 moles) of sodium being added without a permanent blue color developing. The reaction mixture was worked up as before giving 28 mg. of crude petroleum ether-soluble product. This material on chromatography over alumina furnished 15 mg. of white crystals, m.p. 75.5–78.5°. Rechromatography and recrystallization from ethanol gave crystals of m.p. 79–81°, undepressed on admixture with authentic 1′-methyl-1,2-cyclopentenophenanthrene. The infrared (Fig. 3) and ultraviolet absorption spectra of this material are identical with those of 1′-methyl-1,2-cyclopentenophenanthrene.

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(55) R. A. Friedel and M. Orchin, "Ultraviolet Spectra of Aromatic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1951, Fig. 447.

(56) H. McCombie, B. C. Saunders and G. J. Stacey, *J. Chem. Soc.*, 380 (1945).

[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Preparation and Properties of the Isomeric Forms of Cystine and S-Benzylpenicillamine

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RECEIVED MARCH 28, 1957

Racemization of bis-(acetyl)-L-cystine in glacial acetic solution was achieved readily through the action of acetic anhydride at room temperature, and the epimeric mixture of optically inactive bis-(acetyl)-cystine so derived isolated in high yield. The asymmetric hydrolytic action of the L-directed renal acylase I upon this latter derivative, at *p*H 7.5 and 37°, resulted in a mixture of free L-cystine, monoacetyl-*meso*-cystine (acetyl moiety at D-position), and bis-(acetyl)-D-cystine. The marked insolubility of the former compound permitted its direct isolation from the enzymic digest by filtration, while the two latter compounds were acid hydrolyzed to *meso*-cystine and D-cystine, respectively, after their separation and isolation from the filtrate on a Dowex-50 column in the acid phase. Specific rotation values were −211 and +208° (1% in *N* HCl) for the L- and D-enantiomorphs, respectively, whereas the *meso*-cystine was completely devoid of detectable optical activity. In addition, a resolution of S-benzyl-DL-penicillamine was effected *via* the hydrolytic action of hog kidney amidase on the amide derivative at *p*H 8.0. Subsequent separation of the liberated S-benzyl-L-penicillamine from the unhydrolyzed S-benzyl-D-penicillamine amide on an Amberlite XE-64 column, followed by acid hydrolysis of the latter compound, ultimately yielded the pure enantiomorphs with specific rotation values of +91.3 and −90.6° (1% in *N* HCl) for the L- and D-forms, respectively. Infrared spectra for each of the stereoisomeric forms of cystine, in addition to data on the susceptibility of these compounds or certain of their selected derivatives to the enzymic action of L- and D-amino acid oxidase and hog renal acylase I, are presented.

Cystine is a symmetrical diaminodicarboxylic acid which, although hitherto found in nature only as the L-form, may in fact exist as two racemic modifications, one an internally compensated *meso*-form, and the other a mixture of externally compensated isomerides. Since L-cystine may be relatively inexpensively isolated from acid hydrolysates of certain natural materials, *e.g.*, hair or wool, such source has provided the major supply of commercially available cystine. However, the drastic hydrolytic conditions employed in its manufacture might also lead to rather extensive racemization, and commercial samples of "L-cystine" whose specific rotation values differ by as much as thirty or forty degrees from the generally accepted value of −212° (in *N* HCl) have not been uncommon. With the view in mind that certain biochemical studies demand not only L-cystine, but also the D-