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Oxidative Transformations of Indole Alkaloids. I. The Preparation of Oxindoles from Yohimbine; the Structures and Partial Syntheses of Mitraphylline, Rhyncophylline and Corynoxeine[1]

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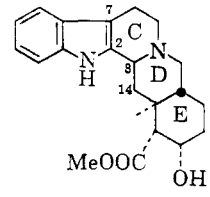
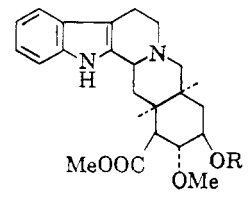
Methanolysis of chloroyohimbine affords an imido ether, which on acid hydrolysis yields a mixture of oxindoles A and B. Performance of this reaction sequence on ajmalicine, corynantheine and dihydrocorynantheidine gives rise to mitraphylline, corynoxeine and rhyncophylline, respectively. Utilizing these and other observations, the detailed stereochemistry of all these oxindole alkaloids was deduced.

The reactivity of the β-position of indoles to electrophilic reagents has long been known.[2] This property has been utilized to effect the transformation of simple indoles into their oxindole and pseudo-indoxyl equivalents *via* the intermediate β-hydroxyindolenines. The latter compounds can be prepared by the reduction of hydroperoxyindolenines (the primary autoxidation product of indoles) or the treatment of indoles with suitable peracids. The reactions of these derivatives of tetrahydrocarbazole have been well studied.[3] Therefore, in principle it should have been possible to transform indole alkaloids themselves into their oxindole and pseudo-indoxyl congeners. This expectation was realized in the iboga alkaloids[4] and in the conversion of cinchonamine into quinamine.[5] This method, however, failed upon application to the tetrahydro-β-carboline alkaloids when reaction with peracids yielded principally the N-oxides.[6] Provided the nitrogen is rendered non-basic, *e.g.*, as in diacetyl tetrahydro-β-carboline itself, the corresponding oxindole and pseudo-indoxyl can be prepared[7] by the hydroxylation method, but it appears difficult to translate this success into a method applicable to an indole alkaloid itself.

To transform the tetrahydro-β-carboline alkaloids into oxindoles, it was therefore necessary to examine the behavior of these alkaloids with other electrophilic reagents. The reaction of yohimbine (Ia) and deserpidine (IIc) with *tert*-butyl hypochlorite had already been described.[8] Deserpidine, for example, yielded a crystalline chloro compound in high yield. Although this product had been assigned an alternative structure by the Danish workers, its infrared and ultraviolet spectra, as well as its facile acid-catalyzed conversion into Δ^3-deserpidine,[9] were consistent only with a β-chloroindolenine structure (IV or VI).[10] Such a system might reasonably be expected to undergo a base-catalyzed[11] rearrangement with elimination of chloride anion and formation of an oxindole.



Ia, C_3-hydrogen α
Ib, C_3-hydrogen β



IIa, C_3-hydrogen α; R = H
IIb, C_3-hydrogen β; R = H
IIc, C_3-hydrogen β; R = 3,4,5-trimethoxybenzoyl

Reaction of *tert*-butyl hypochlorite with yohimbine yielded an amorphous chloroindolenine, probably a mixture of C_7-epimers (IV and VI). On refluxing the crude mixture in methanol containing an equivalent of base, the imido ether VIII was obtained, which upon subsequent hydrolysis in refluxing aqueous acetic acid afforded a mixture of oxindoles, the stronger base of the pair being named yohimbine oxindole B and the weaker one, yohimbine oxindole A.[12] The isolation of two oxindoles was expected since the natural bases are equilibrated to a mixture of the natural and iso bases under conditions[13] similar to those of the hydrolysis. A mechanism for this equilibration has previously been discussed[13,14] (Chart II, IX $\rightleftarrows$ XI $\rightleftarrows$ X) and it required participation of the lone pair electrons on N_b; thus isomerization can be blocked by quaternization. The imido ether VIII therefore could be related stereochemically to the oxindoles *via* its methiodide which on acid hydrolysis gave exclusively yohimbine oxindole A methiodide.

(1) (a) A preliminary account of part of this work has been given, *J. Am. Chem. Soc.*, **84**, 1318 (1962). (b) Presented at the New York–North New Jersey Meeting-in-Miniature, New York, January 22, 1962. (c) See also E. Schlittler and W. I. Taylor, *Experientia*, **16**, 246 (1960).

(2) T. S. Stevens in "Chemistry of Carbon Compounds," E. H. Rodd, Editor, Vol. IVa, Elsevier Press, New York, N. Y., 1957, p. 78.

(3) B. Witkop, *Bull. soc. chim. France*, 423 (1954).

(4) M. F. Bartlett, D. F. Dickel and W. I. Taylor, *J. Am. Chem. Soc.*, **80**, 126 (1958).

(5) B. Witkop, *ibid.*, **72**, 2311 (1950).

(6) *Inter alia*, P. R. Ulshafer, W. I. Taylor and R. H. Nugent, *Compt. rend.*, **244**, 2989 (1957); B. Witkop and S. Goodwin, *J. Am. Chem. Soc.*, **75**, 3371 (1953).

(7) E. E. van Tamelen, K. V. Siebrasse and J. B. Hester, *Chemistry and Industry*, 1145 (1956).

(8) W. O. Godtfredsen and S. Vangedal, *Acta Chem. Scand.*, **10**, 1414 (1956).

(9) This procedure (ref. 8) is an excellent general method for the preparation of Δ^3-derivatives of tetrahydro-β-carboline alkaloids.

(10) Also proposed by J. E. Saxton in "The Alkaloids," R. H. Manske, Ed., Vol. VII, Academic Press, Inc., New York, N. Y., 1960, p. 90.

(11) The reaction also proceeds under weakly acidic conditions; J. Shavel and H. Zinnes, *J. Am. Chem. Soc.*, **84**, 1320 (1962).

(12) The use of the suffixes A and B follows the convention used for the uncarines [T. Nozoye, *Chem. Pharm. Bull.*, **6**, 300 (1958)]. It will emerge from our work that these suffixes correspond to the lactam moiety being α (below) and β (above), respectively, to the plane of the CDE rings.

(13) J. C. Seaton, M. D. Nair, O. E. Edwards and L. Marion, *Can. J. Chem.*, **38**, 1035 (1960).

(14) E. Wenkert, J. H. Udelhofen and N. K. Bhattacharyya, *J. Am. Chem. Soc.*, **81**, 3763 (1959).

The stereochemistry at C_3[15] in the imido ether VIII was most probably the same as in yohimbine (Ia), since rearrangements of this type usually proceed with retention of configuration in the migrating group.[16] This conclusion was supported by the results obtained from the methanolysis of the amorphous mixture of chloroindolenines derived from pseudo-yohimbine (Ib). A trace (4%) of the yohimbine imido ether (VIII) resulted, indicating that extensive racemization had not taken place at either step.

Equilibration studies with yohimbine oxindoles A and B showed the stronger base B (X) predominated after acid treatment, while the weaker A (IX) predominated after reflux in pyridine. The energy difference in both bases is quite small, as both oxindoles are present to a considerable extent in either equilibrium mixture.

To explain the enhanced stability of the conjugate acid of oxindole B (X), models of the four stereochemical possibilities IX, X, XII and XIII were examined. Only in X could the conjugate acid be stabilized by hydrogen bonding to the oxindole carbonyl; furthermore, electrostatic repulsion between the partial charge on the oxygen atom of the carbonyl group and the lone pair orbital of N_b might be expected to destabilize this compound in the free base form. Structure X was therefore assigned to yohimbine oxindole B. Since yohimbine oxindole A has been related stereochemically to the imido ether, which also has the C_3-hydrogen α, oxindole A must be IX.

These assignments received support from examination of the p.m.r. spectra of the isomeric oxindoles. As a consequence of the alignment of the aromatic ring with respect to the carbomethoxy group, there is considerable shielding of the methyl hydrogens in both oxindoles. The signal from these protons appears upfield relative to the corresponding signal of yohimbine by 14 c.p.s. in oxindole A and 13 c.p.s. in oxindole B. The extent of the shielding may be calculated from a knowledge of the time averaged position of methyl hydrogens, expressed in cylindrical coördinates, from the plane of the aromatic ring and its center as origin.[17] Using Dreiding models, the four possible yohimbine oxindoles were arranged in order corresponding to increased shielding of the carbomethoxyl group, *viz.*, X, IX, XII and XIII. As the observed difference in shielding between the isomers is small (1 c.p.s.), they must be represented by structures which are adjacent in this sequence. The p.m.r. spectra of both the imido ether VIII and oxindole A possess a further feature of interest. Between the tetramethylsilane signal and commencement of the fingerprint, there is a two-hydrogen multiplet, which is not present in oxindole B. Therefore oxindole A must possess a shielded methylene group. This can only be at C_{14} and furthermore the aromatic ring must be on the same side of the plane of the CDE rings as the group it is shielding.

(15) The atoms in all compounds discussed are numbered according to their equivalents in yohimbine. Substituents below the plane of the CDE rings are designated α and those above β.

(16) C. K. Ingold in "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 500.

(17) C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).

CHART I

PREPARATION OF OXINDOLES FROM INDOLES

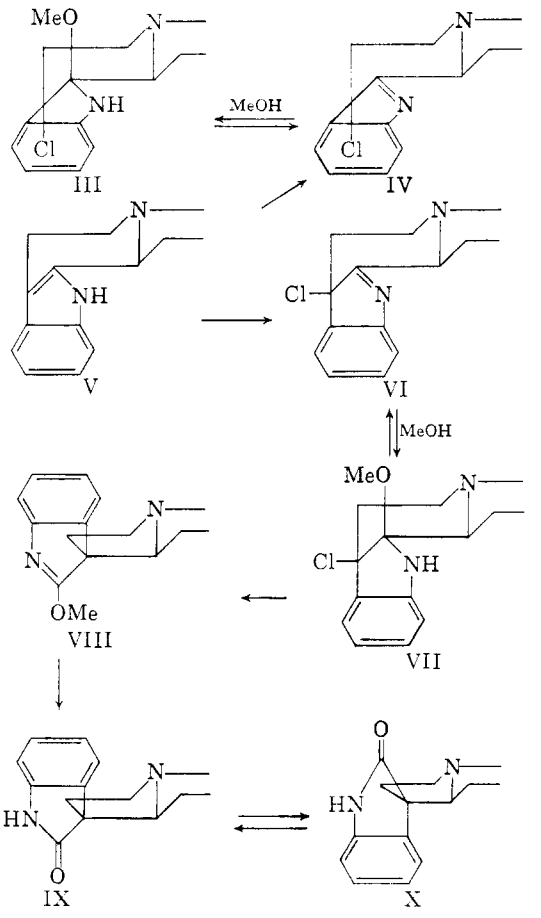
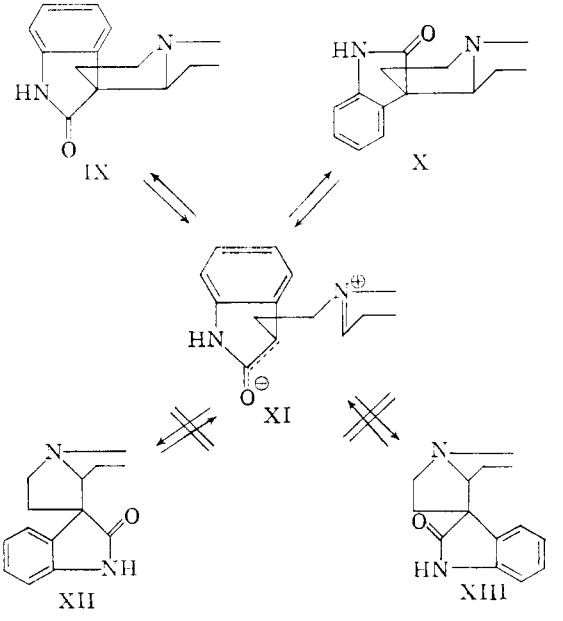


CHART II

INTERCONVERSIONS OF OXINDOLES A AND B



Since oxindole A is known to have the C_3-hydrogen α from its correlation with the imido ether, its structure must be represented by IX and that of

TABLE I

Indole			Equivalent oxindole		
Name	$[\alpha]_D$	pK_a	Name	$[\alpha]_D$	pK_a
Yohimbine	+11°	7.1	Oxindole B	−90°	6.4
			Oxindole A	+57	5.3
Methyl isodeserpidate			Oxindole B	−102	5.3
			Oxindole A	±0	<4
Ajmalicine	−60	6.3	Mitraphylline	−8	5.5
			Isomitraphylline	+18	4.6
Dihydrocorynantheine	+30 (MeOH)	7.2	Rhyncophylline	−15	6.3
			Isorhyncophylline	+8	5.2

oxindole B by X or XII, the former being more likely because of the absence of C_{14}-proton shielding.

Although yohimbine (Ia) had proved to be a satisfactory model for the above transformations, it was considered worthwhile to examine another model which possessed a *cis*-DE, instead of a *trans*-DE ring junction. Chlorodeserpidine, which was previously described,[8] was crystalline and homogeneous, in contrast to chloroyohimbine; furthermore, it appeared to be resistant to methanolysis, except for the expected loss of the trimethoxybenzoyl group.[18] A more suitable model was considered to be methyl isodeserpidate (IIa). Chlorination gave predominantly one compound; methanolysis of the crude mixture gave a very small yield of a crystalline imido ether; hydrolysis of this compound gave a pair of oxindoles.

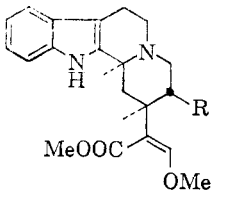
The great difference between the yields of rearranged material from the *trans*-DE case and the *cis*-DE cases may be a reflection of the composition of the chlorination mixture. In the *trans*-DE case, both chloroindolenines are present in equal amounts (proved conclusively in the case of the chloroyohimbanes[11]), but only the equatorially oriented chlorine atom has the correct stereochemistry to permit coplanarity of the four atoms involved in the rearrangement step, *viz.*, C_1, C_7, C_2 and C_3, a situation known to favor rearrangement. In the *cis*-DE case, where one chloroindolenine predominates, the halogen must be axially oriented (IV) since it is stable to methanolysis.

This conclusion received support from an examination of the principal product from the chloromethyl isodeserpidate methanolysis. Although it was still a chloroindolenine, further methanolysis gave no imido ether. On treatment with methanolic hydrogen chloride it was converted, as expected,[8] into methyl Δ^3-deserpidate.

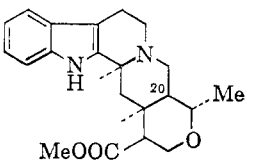
Methyl isodeserpidate oxindole A was too weak a base for a determination of its pK_a in 80% methyl Cellosolve–water, and it did not yield a crystalline methiodide.[19] Since the rate of hydrolysis of the imido ether proved to be greater than the rate of isomerization of the product, oxindole A, the former was correlated with the latter by showing that the hydrolysis product was much richer in oxindole A than the equilibrium mixture.

The pK_a's, molecular rotational differences and R_f differences of methyl isodeserpidate oxindoles A and B paralleled those of yohimbine oxindoles A and B, and they have therefore been assigned analogous structures, IX and X, respectively (see Table I).

With the model studies completed, attention was directed toward those substances which might be transformable into known oxindole alkaloids. Ajmalicine (XVb) and tetrahydroalstonine[20] (XVa) were thought to be related as *cis*-DE and *trans*-DE,



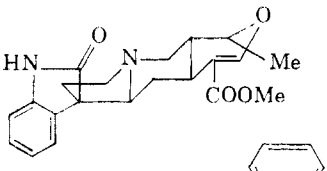
XIVa, R = Et
XIVb, R = CH=CH$_2$



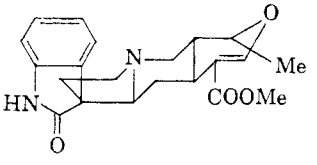
XVa, C_{20}-hydrogen α
XVb, C_{20}-hydrogen β

respectively,[21] at the time of this work. Chlorination of ajmalicine gave an amorphous chloroindolinine, which on methanolysis gave an imido ether in good yield. Tetrahydroalstonine gave a crystalline chloroindolenine in high yield, which was quite stable under methanolysis conditions. In view of the work with model compounds, these observations led to doubts as to the correctness of the stereochemical assignments to these alkaloids. Recent work has shown that these doubts were well justified.[22]

Hydrolysis of the imido ether from chloroajmalicine gave mitraphylline (XVI).[23] By the thin layer chromatography method employed for the methyl isodeserpidate study, the imido ether of



XVI, mitraphylline



XVII, isomitraphylline

(18) Methyl deserpidate (IIb) is actually prepared by methanolysis of deserpidine [H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André and P. R. Ulshafer, *J. Am. Chem. Soc.*, **77**, 4335 (1955)].

(19) These effects may be attributable to the additional 1:3-diaxial interaction, with the lone pair orbital of N_b, present in the *cis*-DE compounds.

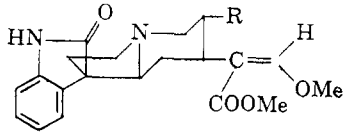
(20) We are indebted to Dr. J. R. Price for a generous gift of alstonine.

(21) E. Wenkert and D. K. Roychaudhuri, *J. Am. Chem. Soc.*, **80**, 1613 (1958). N. Neuss and H. E. Boaz, *J. Org. Chem.*, **22**, 1001 (1957).

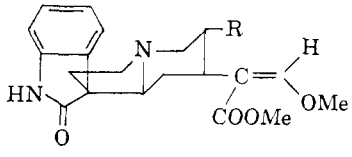
(22) E. Wenkert, B. Wickberg and C. Leicht, *J. Am. Chem. Soc.*, **83**, 5037 (1961); M. Shamma and J. B. Moss, *ibid.*, **83**, 5038 (1961).

(23) We are indebted to Drs. L. Marion and N. Neuss for authentic samples of the alkaloid, and to the latter for X-ray powder diagrams of the natural and synthetic compounds.

ajmalicine was related stereochemically to isomitraphylline. Comparison of the physical properties (p.m.r. spectra, pK_a's, optical rotational differences, chromatographic behavior) of mitraphylline (ajmalicine oxindole B) and isomitraphylline (ajmalicine oxindole A) showed them to be completely analogous to the model A and B oxindoles. Thus the complete structures for mitraphylline and isomitraphylline are XVI and XVII, respectively.



XVIIIa, rhyncophylline, R = Et
XVIIIb, corynoxeine, R = CH=CH$_2$



XIXa, isorhyncophylline, R = Et
XIXb, isocorynoxeine, R = CH=CH$_2$

Chlorination of corynantheine,[24] whose structure has been proved rigorously,[25] gave a chloroindolenine which on methanolysis gave in good yield a glassy imido ether. Hydrolysis of this compound gave an oxindole possessing physical properties comparable with those of corynoxeine[26] which may now be given the detailed structure XVIIIb.

Since hydrogenation[26] of corynoxeine furnished rhyncophylline (XVIIIa), dihydrocorynantheine should be convertible into rhyncophylline; this expectation was realized. The synthetic material was identical in all respects with a natural sample.[27] Comparison of the physical properties of rhyncophylline (dihydrocorynantheine oxindole B) and its iso compound (dihydrocorynantheine oxindole A) showed them to belong to the B and A types, respectively, so that they have the expected structures XVIIIa and XIXa. It should be noted that all these naturally occurring oxindole alkaloids have thus been shown to have the expected[28] absolute stereochemistry at C_{15}.

Since completion of this work, there have been several papers pertaining to the stereochemistry of these oxindole alkaloids. A Japanese group[29] has synthesized N_a-methylrhyncophyllane showing that the groups at C_{15} and C_{20} are *trans* oriented. From an analysis[25] of the p.m.r. spectrum of mitraphylline it has been found that the DE ring junction is *trans* and that the methyl group is α. Both these papers are in complete accord with our findings; however, in a third paper[30] quite different conclusions were reached from a critical examination of the existing literature. This author attempted to deduce unique structures for this type of alkaloid from the scattered observations in the literature and by conformational analysis.

We have several criticisms of his basic postulates. He was unaware that the relative amounts of the oxindoles A and B at equilibrium differed in acidic and basic media. This relationship had been observed with the uncarines, but had not been further commented on.[31] As the model compound[30] used by Hendrickson was purified *via* its hydrochloride which presumably had the B configuration X, the structure assigned to this compound is correct; the error lies in then assuming that this form is also the more stable in the free base form. Finally the author interpreted the basicity difference between the oxindoles A and B as being due to interaction of the N_b lone pair electrons with the oxindole carbonyl group, increased interaction decreasing the basicity of the alkaloids. This effect is important when the basic nitrogen can interact with a ketonic moiety[32] but would be expected to be of little significance with an amide carbonyl group. On the other hand, the hydrogen bond formed between the oxindole carbonyl and the proton in the conjugate acid should be stronger than that formed by a ketone carbonyl. For these reasons, we favor an explanation of the basicity difference in oxindoles A and B in terms of hydrogen bonding or lack of it in the respective conjugate acids.

Acknowledgments.—We are grateful to Dr. E. Schlittler for his constant interest and encouragement. We are indebted to Mr. L. Dorfman and his staff for the microanalytical and physical measurements, and to Mr. B. Korzun and Mr. S. Brody for paper and thin layer chromatograms. We also wish to acknowledge the technical assistance of Mr. N. Geller. Our thanks are due to Dr. James N. Shoolery of Varian Associates for several of the p.m.r. spectra.

Experimental

All melting points are uncorrected. Unless stated, optical rotations were measured in chloroform at 25 ± 2° ($c \approx 1$), ultraviolet spectra in ethanol, infrared spectra in chloroform, proton magnetic spectra (Varian A60 machine) in deuteriochloroform and the dissociation constants in 80% methyl Cellosolve–water. The alumina used in chromatography was Woelm neutral activity III. Silica gel G (Merck, Darmstadt) was used as the absorbent for thin layer chromatography, and results are reported as distance in cm. that the compounds ran from the origin.

Chloroyohimbine.—Yohimbine (1 g.) was dissolved in methylene chloride (40 ml.) and cooled in an ice–salt-bath. Triethylamine (0.4 ml.) was added, and to the well-stirred mixture cooled in ice was added dropwise, *tert*-butyl hypochlorite in carbon tetrachloride (29 ml. of 0.0975 *M* solution, 1 molar equiv.) over 15 minutes. The mixture was stirred for 30 minutes, washed with water, and the solvent removed *in vacuo* below 40°. The resultant yellow foam which could not be crystallized had ν_{max} 1710, 1598 cm.$^{-1}$; λ_{max} 290 mμ.

Methanolysis of Chloroyohimbine.—Chloroyohimbine (3.7 g.) was dissolved in methanol (100 ml.) and 0.43 *N* potassium hydroxide (22 ml., 1 molar equiv.) added. The mixture was refluxed under nitrogen for 1.5 hours, diluted with water and extracted with methylene chloride. Removal of the solvent *in vacuo* gave an orange foam (2.0 g.).

(24) We are grateful to Professor M.-M. Janot for a generous gift of this rare alkaloid.

(25) E. Wenkert, B. Wickberg and C. Leicht, *Tetrahedron Letters*, No. **22**, 822 (1961), and references therein.

(26) N. A. Cu, R. Goutarel and M.-M. Janot, *Bull. soc. chim. France*, 1292 (1957).

(27) Isolated from *Mitragyna stipulosa* by Dr. D. F. Dickel.

(28) E. Wenkert and N. V. Bringi, *J. Am. Chem. Soc.*, **81**, 1474, 6525 (1959).

(29) Y. Ban and T. Oishi, *Tetrahedron Letters*, No. **22**, 791 (1961).

(30) J. B. Hendrickson, *J. Am. Chem. Soc.*, **84**, 656 (1962).

(31) H. Kondo and T. Nozoye, *Ann. Reports ITSUU Lab.*, **1**, 71 (1950).

(32) N. J. Leonard, *Rec. Chem. Prog.*, **17**, 243 (1956).

Chromatography on alumina, and elution by methylene chloride–benzene (3:2) yielded a white crystalline solid (1.25 g.), m.p. 195–197°. Recrystallizations from methanol gave the imido ether VIII, m.p. 198–199°; ν_{max} 1708, 1582 cm.$^{-1}$; $[\alpha]_D$ +109°; λ_{max} 251–257 mμ (log ϵ 3.76).

Anal. Calcd. for $C_{22}H_{28}N_2O_4$: C, 68.72; H, 7.34; N, 7.29. Found: C, 68.92; H, 7.32; N, 7.11.

The methiodide had m.p. 286–289° (darkening); ν_{max}^{Nujol} 1730, 1623, 1592 cm.$^{-1}$; λ_{max} 253 mμ (log ϵ 3.71).

Anal. Calcd. for $C_{23}H_{31}N_2O_4I$: C, 52.48; H, 5.94; N, 5.33. Found: C, 52.15; H, 6.14; N, 5.04.

Yohimbine Oxindole B (X).—The above imido ether (5.6 g.) was dissolved in 10% aqueous acetic acid (560 ml.) and refluxed under nitrogen for 4 hours. The reaction mixture was made basic (2 *N* NaOH) and extracted with methylene chloride. Removal of the solvent gave a residue (3.43 g.) which was chromatographed on alumina. Elution with methylene chloride containing 0.5% methanol gave the crystalline oxindole B (2.5 g.), m.p. 216–217°, which was recrystallized to constant m.p. 222–224°; γ_{max} 1705, 1620 cm.$^{-1}$; $[\alpha]_D$ −9°; λ_{max} 251–254 mμ (log ϵ 3.86); pK_a 6.4.

Anal. Calcd. for $C_{21}H_{26}N_2O_4$: C, 68.08; H, 7.07; N, 7.56. Found: C, 67.75; H, 7.10; N, 7.56.

Yohimbine oxindole B methiodide had m.p. 264–266° from methanol–benzene; ν_{max}^{Nujol} 1740, 1718, 1624 cm.$^{-1}$; $[\alpha]_D$ ± 0° (MeOH); λ_{max} 253 mμ (log ϵ 3.90).

Anal. Calcd. for $C_{22}H_{29}N_2O_4I$: C, 51.57; H, 5.71; N, 5.47. Found: C, 51.75; H, 5.96; N, 5.59.

Yohimbine Oxindole A (IX).—Yohimbine oxindole B (350 mg.) was refluxed overnight in pyridine (5 ml.). The solvent was removed *in vacuo*, and the residue resolved by means of thin layer chromatography using ethyl acetate–chloroform (9:1) as the developing agent. The plates were examined under ultraviolet light, and lines, corresponding to the boundaries of maximum concentration of the thus detected bands, were scratched parallel to the solvent front. In this way seven zones, numbered according to increasing R_f, were separately removed and eluted with methanol–methylene chloride.

No. of zone	Wt., mg.	Ultrav., mμ	State
1	21.5	None	Amorph.
2	15.3	None	Amorph.
3	9.9	280, 252	Cryst.
4	104.4	280, 252	Cryst.
5	62.7	280, 252	Cryst.
6	144.1	280, 252	Cryst.
7	14.5	280, 252	Amorph.

Zone 4 was identified as yohimbine oxindole B. Zones 5 and 6 were combined and crystallized from ethyl acetate to furnish yohimbine oxindole A, m.p. 168–170°; ν_{max} 1714, 1623 cm.$^{-1}$; $[\alpha]_D$ +59°; λ_{max} 251–264 mμ (log ϵ 3.83); pK_a 5.3.

Anal. Calcd. for $C_{21}H_{26}N_2O_4$: C, 68.08; H, 7.07; N, 7.56. Found: C, 68.24; H, 7.31; N, 7.35.

Yohimbine oxindole A methiodide had m.p. 222–226° and $[\alpha]_D$ +81° (methanol) after crystallization from methanol–isopropyl ether. This was the sole product from the hydrolysis of the imido ether methiodide. The methiodide analyzed poorly; it was converted to the methochloride [Amberlite IRA 400 (Cl^-)], m.p. 210–215° dec., $[\alpha]_D$ +84° (methanol).

Anal. Calcd. for $C_{22}H_{29}N_2O_4Cl\cdot H_2O$: C, 60.19; H, 7.12; N, 6.38. Found: C, 59.87; H, 7.41; N, 5.97.

Acid Equilibration of Yohimbine Oxindole A.—Yohimbine oxindole A (10 mg.) was refluxed in 10% aqueous acetic acid (5 ml.) for 1 hour. The solution was made basic (2 *N* NaOH) and extracted by methylene chloride. The product was subjected to thin layer chromatography, using ethyl acetate–chloroform (9:1) as the developing agent, and found to consist of the two oxindoles with the B-form predominating.

Chloro-pseudo-yohimbine.—Pseudo-yohimbine (500 mg.) was dissolved in methylene chloride (70 ml.), cooled in an ice–salt-bath, and triethylamine (0.2 ml.) added. To the well-stirred mixture was added dropwise a cold solution of *tert*-butyl hypochlorite in carbon tetrachloride (15.7 ml. of 0.09 *M*, 1 molar equiv.). After addition was completed, the mixture was washed with water, and taken to dryness *in vacuo* to yield a brown foam (574 mg.); ν_{max} 1710, 1590 cm.$^{-1}$; λ_{max} 288 mμ.

Methanolysis of Chloro-pseudo-yohimbine.—Chloro-pseudo-yohimbine (574 mg.) was dissolved in methanol (20 ml.) and aqueous potassium hydroxide added (1.6 ml. of 0.852 *N*, 1 mole equiv.). The mixture was refluxed under nitrogen for 1.5 hours, diluted with water and methylene chloride extracted. The solvent was removed and the residue (407 mg.) after chromatography on alumina yielded, from the methylene chloride–benzene (2:3) eluate, the imido ether (26 mg.) derived from yohimbine. Further elution of the column with methylene chloride containing methanol (5%) afforded the principal band, a resin with λ_{max} 365 mμ.

Methyl Isodeserpidate (IIa).—Methyl deserpidate (1 g.) was chlorinated by the *tert*-butyl hypochlorite method (in a manner similar to the above preparations) to yield a brown foam (ν_{max} 1730, 1590 cm.$^{-1}$; λ_{max} 290 mμ).

The entire product was dissolved in methanolic hydrogen chloride solution and refluxed for a few minutes. The solvent was removed and the residue dissolved in water. An aqueous solution of sodium borohydride (5%) was added slowly with stirring until the color of the solution was discharged. The cream-colored precipitate was extracted into methylene chloride which was dried and concentrated to dryness. The resulting crude methyl isodeserpidate (867 mg.), according to the paper chromatographic results (chloroform-10% pyridine on formamide-impregnated paper), was essentially homogeneous (R_f 0.64) and free from methyl deserpidate (R_f 0.85). All attempts to crystallize the free base failed, but it could be characterized as its nitrate, needles from methanol, m.p. 266–268° dec., ν_{max} 1711 cm.$^{-1}$, $[\alpha]_D$ −35°.

The Imido Ether from Methyl Isodeserpidate.—Methyl isodeserpidate (500 mg.) was converted into its chloro derivative upon treatment with *tert*-butyl hypochlorite. The chloro compound was amorphous but had the correct spectral properties; ν_{max} 1739, 1594 cm.$^{-1}$; λ_{max} 285 mμ. The crude derivative was refluxed for 1.5 hours in methanol (300 ml.) and 0.42 *N* potassium hydroxide (30 ml.). The reaction mixture was diluted with water and extracted into methylene chloride. The organic phase was dried and concentrated to dryness; the residue (442 mg.) in methylene chloride was purified by filtration through alumina, and the tail fractions crystallized. Crystallization of this material (89 mg.) from ether gave the imido ether, m.p. 183°; ν_{max} 1734, 1620, 1584 cm.$^{-1}$; $[\alpha]_D$ +56°; ν_{max} 253–254 mμ (log ϵ 3.75).

Anal. Calcd. for $C_{23}H_{30}N_2O_5$: C, 66.64; H, 7.30; N, 6.76. Found: C, 66.71; H, 7.32; N, 6.61.

Methyl Isodeserpidate Oxindoles A and B.—The crude product (500 mg.) from a further methanolysis of chloromethyl isodeserpidate was refluxed for 45 minutes in 10% aqueous acetic acid (50 ml.). The solution was basified and extracted with methylene chloride, dried and concentrated to dryness. A portion (277 mg.) of this material (477 mg.) was chromatographed on alumina. The 0.5% methanol in methylene chloride eluate furnished methyl deserpidate oxindole A (40 mg.), m.p. 230–231° from ether; ν_{max} 1726, 1710, 1624 cm.$^{-1}$; $[\alpha]_D$ ± 0°; $pK_a < 4$.

Anal. Calcd. for $C_{22}H_{28}N_2O_5$: C, 65.98; H, 7.05; N, 7.00. Found: C, 65.60; H, 7.14; N, 6.64.

Further elution of the column by 5% methanol in methylene chloride afforded methyl isodeserpidate oxindole B, crystals from isopropyl ether–methylene chloride, m.p. 226°; ν_{max} 1720, 1624 cm.$^{-1}$; $[\alpha]_D$ −102°; λ_{max} 252 mμ (log ϵ 3.87); pK_a 5.3.

Anal. Calcd. for $C_{22}H_{28}N_2O_5$: C, 64.52; H, 7.13; N, 6.84. Found: C, 64.64; H, 7.11; N, 6.77.

Both methyl isodeserpidate oxindoles were dissolved in benzene containing methyl iodide and heated in sealed tubes at 100°, but no crystalline methiodides could be obtained.

Equilibration of Methyl Isodeserpidate Oxindoles A and B.—Methyl isodeserpidate oxindoles A (10 mg.) and B (10 mg.) were separately refluxed in 10% aqueous acetic acid (6 ml.) for 8 hours. The solution was made basic and extracted by methylene chloride. The product from each was assayed by thin layer chromatography [developing agent, ethyl acetate-chloroform (4:1)]. Both oxindoles

gave rise to the same equilibrium mixture of oxindole B (1.5 cm.) and A (5.5 cm.) in an approximate ratio of 2:1.

Acid Hydrolysis of the Imido Ether Derived from Chloromethyl Isodeserpidate.—The imido ether (10 mg.) was refluxed in aqueous acetic acid (6 ml.) for 30 minutes. The product of the reaction no longer possessed the infrared ν_{max} 1584 cm.$^{-1}$, characteristic of the imido ether, and upon assay by thin layer chromatography was shown to consist of about equal amounts of methyl deserpidate oxindoles A and B.

Chloromethyl deserpidate was prepared from methyl deserpidate (1.4 g.) by the same procedure used with methyl isodeserpidate. The crude chloro compound was amorphous and had ν_{max} 1728, 1592 cm.$^{-1}$ and λ_{max} 282 mμ.

Methanolysis of Chloromethyl Deserpidate.—The crude chloro compound from the above experiment was refluxed for 1.5 hours in methanol (900 ml.) and 0.42 *N* potassium hydroxide (90 ml.). Dilution with water, extraction with methylene chloride, and removal of the solvent *in vacuo* yielded a residue (1.05 g.). Chromatography on alumina and elution by methylene chloride furnished some crystalline fractions (160 mg.) which upon recrystallization from ether afforded the same imido ether as derived above from chloromethyl isodeserpidate.

Further elution of the column with 5% methanol in methylene chloride gave recovered chloromethyl deserpidate (578 mg.), λ_{max} 260 mμ, shoulder 282 mμ, changing to λ_{max} 365 and 252 mμ upon warming in methanolic hydrogen chloride.

Anal. Calcd. for $C_{22}H_{27}O_4N_2Cl$: Cl, 8.46. Found: Cl, 6.54.

The recovered chloro compound (200 mg.) was subjected once again to the above methanolysis conditions and again about 70% of the starting material was recovered.

The amorphous compound (100 mg.) was allowed to stand for 2 days in pyridine (2 ml.) in the presence of trimethoxybenzoyl chloride. The pyridine was removed *in vacuo* and the residue chromatographed over alumina. The principal band (60 mg.) was found in the 0.5% methanol in methylene chloride eluate. Although it could not be crystallized, paper chromatographically the product consisted principally of chlorodeserpidine.

Chloroajmalicine.—Triethylamine (4 ml.) was added to ajmalicine (9.46 g.) in methylene chloride (400 ml.) and the mixture cooled in an ice–salt-bath. One mole equivalent of *tert*-butyl hypochlorite in carbon tetrachloride (143.5 ml. of 0.187 *M*) was added dropwise during 30 minutes. After 30 additional minutes the organic phase was washed with water and concentrated to dryness *in vacuo*. The resultant chloro compound (8.67 g.) was amorphous, but had the expected spectral properties, γ_{max} 1700, 1620, 1598 cm.$^{-1}$, λ_{max} 288 mμ.

Methanolysis of Chloroajmalicine.—Chloroajmalicine (1 g.) was dissolved in methanol (50 ml.) and 0.437 *N* potassium hydroxide (11.75 ml., 2 molar equiv.) was added. The mixture was refluxed for 2 hours, diluted with water and extracted into methylene chloride. Removal of the solvent gave a residue (556 mg.) which was chromatographed over alumina. The methylene chloride eluate furnished the crystalline imido ether (396 mg.) which was recrystallized from aqueous methanol; m.p. 196–197°; ν_{max} 1700, 1624, 1586 cm.$^{-1}$; $[\alpha]_D$ +79°; λ_{max} 239–240° (log ϵ 4.19).

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 69.10; H, 6.85; N, 7.32; OMe, 16.23. Found: C, 69.03; H, 6.85; N, 7.09; OMe, 15.86.

Mitraphylline (XVI).—The above imido ether (250 mg.) was refluxed in 10% aqueous acetic acid (25 ml.) for 4 hours. The solution was made basic and extracted with methylene chloride. The product (220 mg.) upon crystallization from methanol gave mitraphylline (132 mg.), m.p. 271–272°, $[\alpha]_D$ −8°, −38° (0.1 *N* HCl), pK_a 5.5, identical in all respects with natural mitraphylline.[33]

Isomitraphylline (XVII).—Mitraphylline (50 mg.) was refluxed overnight in pyridine (8 ml.), concentrated to dryness *in vacuo* and the residue chromatographed over alumina. Elution by 1% methanol in methylene chloride furnished two fractions; the slower moving (10 mg.) was mitraphylline, whereas the faster running material (40 mg.) was amorphous. It was homogeneous according to thin layer chromatography and had $[\alpha]_D$ +18° and a pK_a 4.5.

(33) J. C. Seaton, R. Tondeur and L. Marion, *Can. J. Chem.*, **36**, 1031 (1958).

Acid Equilibration of Isomitraphylline.—The base (28 mg.) was refluxed for 1 hour in 10% aqueous acetic acid (5 ml.), poured into excess 10% potassium bicarbonate and extracted with methylene chloride. Removal of the solvent gave a clear glass (24 mg.) which furnished mitraphylline (13 mg.), m.p. 269–270° after crystallization from methanol. Examination of the crystallization mother liquors by thin layer chromatography showed them to be a 50:50 mixture of mitraphylline and its iso derivative.

Acid Hydrolysis of the Imido Ether Derived from Ajmalicine.—The imido ether (10 mg.) was refluxed in 10% aqueous acetic acid (7 ml.). Aliquots (0.75 ml.) were removed at specified intervals of time and examined by thin layer chromatography (ethyl acetate–chloroform, 9:1, used as the developing agent); mitraphylline and isomitraphylline ran 5.0 and 10.5 cm., respectively.

Time of removal of aliquot, min.	5	30	120	360
Mitraphylline/ Isomitraphylline	0.5	1.0	4.0	4.0

Chlorocorynantheine.—*tert*-Butyl hypochlorite (10.2 ml. of 0.51 *M*) was added dropwise over 30 minutes to a stirred cooled solution of corynantheine (200 mg.) and triethylamine (0.1 ml.) in methylene chloride (50 ml.). The solution was washed with water, dried and concentrated to dryness. The amorphous chloro derivative has γ_{max} 1700, 1640, 1596 cm.$^{-1}$ and λ_{max} 295 mμ.

Corynoxeine (XVIIIb).—Crude chlorocorynantheine (200 mg.) was refluxed for 1 hour in methanol (20 ml.) and 0.42 *N* potassium hydroxide (0.6 ml.), diluted with water and extracted with methylene chloride. Removal of the solvent gave a residue (174 mg.) which after chromatography on alumina using benzene as eluent gave the glassy imido ether (120 mg.); ν_{max} 1700, 1644, 1584 cm.$^{-1}$; and λ_{max} 244 mμ.

The above imido ether (180 mg.) was refluxed for 1.5 hours in 10% aqueous acetic acid, poured into excess 10% potassium bicarbonate and extracted with methylene chloride. Removal of the solvent afforded a glass which partially crystallized on contact with methanol. Recrystallization from acetone gave corynoxeine (104 mg.), m.p. 212–214°; ν_{max} 1710, 1646, 1626 cm.$^{-1}$; λ_{max} 242–245 mμ (log ϵ 4.27).

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 69.10; H, 6.85; N, 7.32. Found: C, 69.15; H, 7.05; N, 7.35.

Rhyncophylline (XVIIIa).—(a) *tert*-Butyl hypochlorite in carbon tetrachloride (15 ml. of 0.51 *M*) was added dropwise with stirring over 30 minutes to dihydrocorynantheine (300 mg.) and triethylamine (0.15 ml.) in methylene chloride (75 ml.). Fifteen minutes later it was washed with water, and reduced to dryness to yield the crude chloro compound, γ_{max} 1696, 1640, 1596 cm.$^{-1}$; λ_{max} 296 mμ). This was refluxed for 1.5 hours in methanol (100 ml.) and 0.42 *N* potassium hydroxide (3 ml.), diluted with water and extracted with methylene chloride. The product (285 mg.) was chromatographed on alumina. Benzene eluted the glassy imido ether (260 mg.; ν_{max} 1694, 1640, 1582 mμ; λ_{max} 288 mμ) which could not be induced to crystallize.

The above imido ether was refluxed in 10% aqueous acetic acid (20 ml.) for 1 hour. The solution was made basic and extracted with methylene chloride. The product was partially crystalline and yielded from acetone, crude rhyncophylline (96 mg.), m.p. 208–211°, which gave the pure alkaloid from methanol, m.p. 212–213°; ν_{max} 1710, 1622 cm.$^{-1}$; $[\alpha]_D$ −32°; λ_{max} 243–245 mμ (log ϵ 4.25); pK_a 6.4.

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 68.72; H, 7.34; N, 7.29. Found: C, 68.41; H, 7.38; N, 7.09.

(b) Synthetic corynoxeine (10 mg.) was hydrogenated in methanol using as catalyst palladium on barium sulfate. The product which failed to crystallize was nevertheless shown to contain rhyncophylline by thin layer chromatography.

Isorhyncophylline (XIXa).—Rhyncophylline (300 mg.) was refluxed overnight in pyridine (5 ml.), and concentrated to dryness *in vacuo*. The residue was resolved using thin layer chromatography; the faster moving band was eluted (methanol and methylene chloride) from the plate to yield the amorphous isorhyncophylline (215 mg.), ν_{max} 1706 and 1620 cm.$^{-1}$, $[\alpha]_D$ +14°, λ_{max} 243 (log ϵ 4.27), pK_a 5.3.

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 68.72; H, 7.34; N, 7.29. Found: C, 68.36; H, 7.29; N, 7.14.

Acid Equilibration of Rhyncophylline.—Rhyncophylline (25 mg.) was refluxed overnight in pyridine (5 ml.) and 10% aqueous acetic acid, respectively, and assayed by thin layer chromatography (ethyl acetate–chloroform, 9:1, being the developing agent). Under the conditions used, rhyncophylline traveled 5.0 cm. whereas the iso derivative ran 11.5 cm.

Equilibration conditions	Acidic	Basic
Rhyncophylline/ Isorhyncophylline	2.0	0.5

Chlorotetrahydroalstonine.—*tert*-Butyl hypochlorite (5.3 ml. of 0.975 *M*) was added dropwise over 30 minutes to tetrahydroalstonine (250 mg.) in methylene chloride (15 ml.) containing triethylamine (0.1 ml.). After an additional 30 minutes, the solution was washed with water, taken to dryness and the crystalline residue was recrystallized from methanol to give the chloro derivative, m.p. 194–196° dec.; ν_{max} 1698, 1630, 1596 cm.$^{-1}$; $[\alpha]_D$ +81°; λ_{max} 225–227 mμ (log ε 4.45), 242 mμ (log ε 4.11).

Anal. Calcd. for $C_{21}H_{23}N_2O_3Cl$: C, 65.16; H, 5.99; N, 7.24; Cl, 9.17. Found: C, 64.91; H, 6.22; N, 7.11; Cl, 9.35.

The chloro compound was recovered in 85% yield after attempted methanolysis under conditions used successfully elsewhere in this paper.

Examination of the Alkaloidal Mother Liquors from Mitragyna stipulosa.—The mother liquors from which rhyncophylline had first been crystallized[27] were examined by paper chromatography using the solvent system benzene–chloroform, 1:1, plus 2% pyridine. Among other spots (R_f's 0.0, 0.08, 0.13, 0.20, 0.25, 0.35), rhyncophylline (R_f 0.55) and isorhyncophylline (R_f 0.85) were present, but no spot corresponding to corynoxeine (R_f 0.70) was seen.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUQUESNE UNIVERSITY, PITTSBURGH, PENNSYLVANIA]

New Substrates, New Inhibitors and the Stereochemistry of the Succinic Dehydrogenase System[1]

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L-Chlorosuccinate and L-methylsuccinate are substrates for succinic dehydrogenase, chlorofumarate and mesaconate being produced, respectively, by enzyme catalyzed dehydrogenation. The respective enantiomorphs, D-chlorosuccinate and L-methylsuccinate, are inhibitors of the enzyme and the stereospecificity of succinic dehydrogenase is thereby established. The enzyme exhibits a *trans* stereoselectivity, dehydrogenating *erythro*-3-deuterio-L-chlorosuccinate to 3-deuterio-chlorofumarate. From the above facts the stereochemistry of the behavior of succinic acid in the enzyme substrate complex is deduced. K_M values and K_I values for the new substrates under specified conditions are presented and E_0' values at pH 7.3, 34° for the chlorosuccinate/chlorofumarate and methylsuccinate/mesaconate half-cells are 0.0462 volt and −0.0018 volt, respectively.

In a preliminary communication[3] we have shown that succinic dehydrogenase acts in a stereospecific manner by demonstrating that L-chlorosuccinate and L-methylsuccinate are substrates for the enzyme whereas the corresponding D-enantiomorphs are inhibitors. The purposes of the present paper are to elaborate on the details of the above and related experiments, to report oxidation-reduction potentials for the new substrates and to demonstrate that the dehydrogenation effected by succinic dehydrogenase proceeds in a *trans* manner.[4] The nature, *cis* or *trans*, of the dehydrogenation was investigated with synthetic erythro-deuterio-L-chlorosuccinate as a substrate. This stereospecifically labelled substrate on submission to the action of succinic dehydrogenase yielded chlorofumaric acid containing deuterium, thus demonstrating the *trans* nature of the dehydrogenation.

The work reported herein is also an extension of previous studies[5] on the stereochemistry of Krebs cycle reactions and in this connection was undertaken to explore the stereochemistry of the succinic dehydrogenase system, it being conjectured for several reasons that this system would exhibit both stereospecificity and stereoselectivity. Our reasons for so conjecturing were the facts, that, in general, enzymes do exhibit stereospecificity and stereoselectivity, that succinic dehydrogenase exhibited a very limited substrate range[6] and that retention of deuterium in fumarate on oxidation of deuteriated succinate, the deuteriated succinate having been obtained by anaerobic exchange,[9] could be rationalized on the basis of a *trans* stereoselectivity.[10]

The α-substituted succinic acids were chosen for investigation to constrain the enzyme to demonstrate stereospecificity (in the D and L sense) for the α-hydrogen and because enzyme stereoselectivity (in the *cis* and *trans* sense) could be investigated readily with either a *threo* or *erythro*-3-monodeuterio-α-substituted succinic acid.

Experimental

DL-Chlorosuccinic Acid.—This compound was synthesized from DL-malic acid by treatment with phosphorus pentachloride according to the procedure of Walden.[11] After recrystallization from acetic acid and then from acetone-benzene, the product melted at 151–153°, lit.,[12] 151.5–152°.

D-(+)-Chlorosuccinic Acid.—This compound was prepared from L-malic acid by the above procedure. The recrystallized acid melted 170–173° and its optical rotation

(1) Abstracted in part from the Ph.D. Thesis, August, 1961 of T. P. Fondy and the Master's Thesis, August, 1961, of M. M. Bechtold.

(2) National Science Foundation Coöperative Graduate Fellow.

(3) O. Gawron, A. J. Glaid, III, T. P. Fondy and M. M. Bechtold, *Nature*, **189**, 1004 (1961).

(4) During the course of our work, T. T. Chen and H. van Milligan, *J. Am. Chem. Soc.*, **82**, 4115 (1960), showed that succinate is dehydrogenated in a trans fashion by succinic dehydrogenase.

(5) O. Gawron, A. J. Glaid, III, and T. P. Fondy, *J. Am. Chem. Soc.*, **83**, 3634 (1961).

(6) At the start of this investigation, it was known that racemic methylsuccinic acid slowly decolorized methylene blue in the presence of a heart preparation,[7,8] albeit the product of the reaction, methylfumaric acid, had not been identified.

(7) T. Thunberg, *Ber.*, **258**, 48 (1933).

(8) W. Franke and D. Siewardt, *Z. physiol. Chem.*, **280**, 76 (1944).

(9) S. Englard and S. P. Colowick, *J. Biol. Chem.*, **221**, 1019 (1956).

(10) For an extensive discussion on this point see H. R. Levy, P. Talalay and B. Vennesland, "Progress in Stereochemistry," in press.

(11) P. Walden, *Ber.*, **26**, 214 (1893).

(12) R. Anschütz and C. Bennert, *ibid.*, **15**, 642 (1882).