minutes; after cooling for several hours the crystals were filtered and washed with a little ethanol. The solid (59 g.) was recrystallized four times from ethanol, using 20 ml. of solvent per gram of salt. It then weighed 22.1 g. (61.2%) and had a constant melting point of 158.5–159°, $[\alpha]^{21}D$ −87.5° (*c* 4.375 in pyridine).

Anal. Calcd. for $C_{28}H_{37}O_9N$: C, 63.26; H, 7.02; N, 2.63. Found: C, 63.22; H, 7.11; N, 2.59.

***l*-Dimethylamino-1-octanol-2.**—A suspension of the resolved dibenzoyltartrate (21.9 g.) in 100 ml. of water was treated with 50 ml. of 2 *N* potassium hydroxide, saturated with sodium chloride, and extracted with ether. The extracts were washed with saturated salt solution, dried over potassium hydroxide pellets, and evaporated. The residue was taken up in benzene and distilled; the base was collected at 115–117° (23 mm.) and weighed 5.0 g. (70%), $[\alpha]^{21}D$ −15.3° (neat). The *d*-base is reported[8] to boil at 99–100.5° (11 mm.), with $[\alpha]D$ +12.2°.

Anal. Calcd. for $C_{10}H_{23}NO$: C, 69.30; H, 13.38; N, 8.09. Found: C, 69.19; H, 13.44; N, 8.33.

***d*-Octene-1,2-oxide.**—Excess methyl iodide was added to an ethereal solution of the *l*-amine. An exothermic reaction resulted in an immediate precipitate. After standing overnight, the colorless methiodide was collected and washed with ether. An aqueous solution of 8.1 g. of methiodide was converted to the quaternary hydroxide by passing it through a column of 15 g. of Amberlite IRA-400 resin on the hydroxide cycle and eluting the column with water until the washings were neutral. The eluates were evaporated almost to dryness under reduced pressure below 60°.

The residue was heated at atmospheric pressure; trimethylamine was evolved and an oily liquid steam distilled with the water present. More water was added in small portions and heating continued until no more organic material distilled. The distillate, collected in an ice-bath, was extracted with ether, and the extracts dried over magnesium sulfate and concentrated. Distillation of the residue yielded 1.61 g. (49%) of colorless epoxide, b.p. 60–62° (15 mm.), $[\alpha]^{21}D$ +14.5° (*c* 3.62 in ethanol). Späth reports, for the *l*-oxide,[8] b.p. 60–70° (17 mm.), $[\alpha]^{17}D$ −12.2°. The infrared spectrum of the *d*-oxide was identical with that of the *d,l*-oxide, and showed no evidence of any ketonic product.

Anal. Calcd. for $C_8H_{16}O$: C, 74.92; H, 12.59. Found: C, 74.76; H, 12.32.

***d*-Octanol-2.**—To a stirred, refluxing solution of 1.5 g. of lithium aluminum hydride in 100 ml. of ether was added dropwise a solution of 1.0 g. of *d*-octene-1,2-oxide in 50 ml. of ether. Reflux and stirring were continued for three hours, the mixture cooled and treated cautiously with saturated salt solution, and enough dilute hydrochloric acid added to dissolve the salts. After separating the layers, the aqueous layer was extracted several times with ether. The combined ethereal solutions were washed with water, dried over magnesium sulfate, and concentrated. The residue distilled at 80–82° (17 mm.), and weighed 0.88 g. (86%), $[\alpha]^{21}D$ +10.1° (*c* 5.575 in ethanol); lit.[17] b.p. 86° (20 mm.). $[\alpha]^{17}D$ +9.9°. The infrared spectrum was identical with that of authentic *d,l*-octanol-2.

The acid phthalate, crystallized once from aqueous acetic acid, melted at 74.5–75°, alone or mixed with an authentic sample of *d*-octanol-2-phthalate; lit.[17] m.p. 75°.

Anal. Calcd. for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97. Found: C, 69.20; H, 7.92.

(17) J. Kenyon, "Organic Syntheses," Coll. Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1932, p. 418.

PRINCETON, NEW JERSEY

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

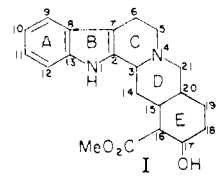
Oxidation–Reduction Studies in the Realm of Indole Alkaloids[1,2]

BY ERNEST WENKERT AND D. K. ROYCHAUDHURI

RECEIVED OCTOBER 21, 1957

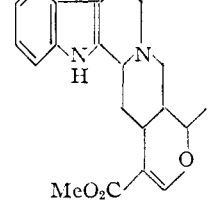
Palladium–maleic acid dehydrogenation is shown to be a general method for the oxidation of ring C of various indole alkaloids and their derivatives. Relative rate data for this process can be used as a diagnostic tool for determination of the stereochemistry of alkaloid ring skeletons. Catalytic hydrogenation and sodium borohydride reduction of tetradehydro compounds lead mainly to *normal* and *allo* products. Reduction–oxidation investigations on sempervirine are described. An infrared spectrophotometric method for the determination of the steric configuration of C-3 in indole alkaloids is presented.

As part of a study of the steric interrelationships of indole alkaloids it was of interest to devise a method of general applicability for the elucidation of the stereochemistry of the ring skeletons of the yohimbine- (I), ajmalicine- (II) and corynantheine-type (III) natural products.



I

The vast body of experimental data in the field of indole alkaloids reveals several procedures for ascertaining the C-3 configuration, albeit a few of these await a test of universality. Catalytic hydrogenation at *p*H 10 of two tetradehydro compounds (IV) of D/E *trans* ring juncture, tetradehydroyohimbine[3] and tetradehydroyohimbane,[4] has yielded *normal* (V) products while hydrogenation of



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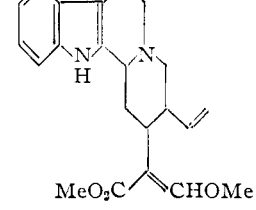


III

d,l-tetradehydroalloyohimbane, a compound of D/E *cis* ring juncture, led to an *allo* (VI) compound.[5] Sodium borohydride reduction of a few

(1) For preliminary communications of this work see (a) E. Wenkert and D. K. Roychaudhuri, THIS JOURNAL, **78**, 6417 (1956); (b) **79**, 1519 (1957).

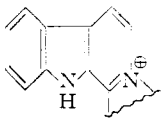
(2) Part of this research was presented to the 17th Midwest Regional Meeting of the American Chemical Society, November 8–9, 1956, Ames, Iowa.

(3) B. Witkop, *Ann.*, **554**, 83 (1943).

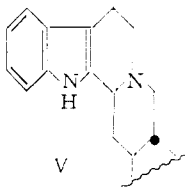
(4) M.-M. Janot, R. Goutarel, A. LeHir, M. Amin and V. Prelog, *Bull. soc. chim. France*, 1085 (1952).

(5) A. LeHir, M.-M. Janot and R. Goutarel, *ibid.*, 1027 (1953).

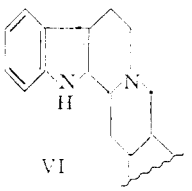
isolated tetradehydro cases has shown similar stereoselectivity.[6-9]



IV

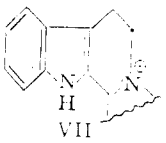


V



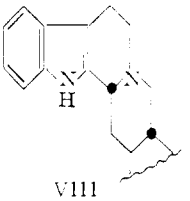
VI

Catalytic hydrogenation and sodium borohydride reduction of Δ^3-dehydro compounds (VII) have led to like results.[10,11] The mercuric acetate method of formation of these compounds has been shown itself to be a sterically differentiating process.[12] The acid-catalyzed or acetic anhydride-induced C-3 equilibration of D/E *cis* systems has been of diagnostic value.[13] Finally, molecular rotation difference data between tetradehydro compounds (IV) and their hydro precursors[14,15] as well as between Δ^3-systems (VII) and the latter[11] have been used to portray the C-3 configuration of various alkaloids.[16]

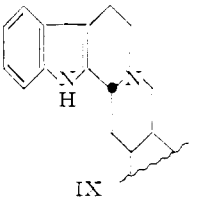


VII

As first task it was of interest to discover whether acid- or acetic anhydride-catalyzed C-3 equilibration,[17] which had been applied so elegantly to the D/E *cis*-rauwolfia alkaloids,[18] could be used also for D/E *trans* systems. As a consequence ψ-yohimbine (VIII) was exposed to a hydrobromic–acetic acid mixture. The reaction yielded yohimbine. An attempted platinum-induced isomerization, however, was unsuccessful.[18] *While an acid-catalyzed C-3 equilibration thus appears to be general, it unfortunately is not of stereo-diagnostic value by itself. Whereas isomer formation in the D/E trans cases means unambiguously conversion of a pseudo (VIII) compound into a normal (V) product, such differentiation between the energetically similar epiallo (IX) and allo (VI) systems is entirely a function of the ring E substituents.*[13]



VIII



IX

Mercuric acetate oxidation of two ketones was attempted. Yohimbone (V) was transformed into Δ^3-dehydroyohimbone (VII), whereas epialloyohimbone resisted oxidation under the experimental conditions employed.[10] As already illustrated previously,[10] reduction of Δ^3-products is stereoselective. *Catalytic hydrogenation or sodium borohydride reduction of 3-dehydro products leads exclusively to normal (V) or allo (VI) compounds.* Interesting applications of this principle have been made most recently on ring A oxygenated alkaloids of the ajmalicine (II) variety.[11]

In view of a need of *pseudo* compounds for purposes to be discussed below, the recently reported method of reduction of Δ^3-dehydro systems by zinc and acid[12] was applied to various derivatives of indole alkaloids. Every reduction led to a mixture of *normal* and *pseudo* products. Among products of special interest there can be cited ψ-yohimbone (X), ψ-yohimbane (XI) and 3-isoajmalicine (II). The formation of X and XI constitutes a formal total synthesis of these compounds in view of the previous synthesis of yohimbone and its ready conversion to yohimbane.[16a] The physical properties of the ajmalicine epimer prove it to be a new compound, not identical with any naturally occurring stereoisomer or ajmalicine. ψ-Yohimbyl alcohol (XII), yet another compound needed for further study, was obtained by lithium aluminum hydride reduction of ψ-yohimbine.

Most recently[19] a procedure of catalytic hydro-

(6) F. E. Bader, D. F. Dickel, C. F. Huebner, R. A. Lucas and E. Schlittler, THIS JOURNAL, **77**, 3547 (1955).

(7) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André and P. R. Ulshafer, *ibid.*, **77**, 4335 (1955).

(8) A. Chatterjee and S. K. Talapatra, *Science and Culture*, **20**, 568 (1955).

(9) T. Wieland and E. Neeb, *Ann.*, **600**, 161 (1956).

(10) E. Wenkert and D. K. Roychaudhuri, *J. Org. Chem.*, **21**, 1315 (1956), and references contained therein.

(11) C. Djerassi, J. Fishman, M. Gorman, J. P. Kutney and S. C. Pakrashi, THIS JOURNAL, **79**, 1217 (1957).

(12) F. L. Weisenborn and P. A. Diassi, *ibid.*, **78**, 2022 (1956).

(13) *Cf.* R. E. Woodson, Jr., H. W. Youngken, E. Schlittler and J. A. Schneider in "Rauwolfia," Little Brown and Co., Boston, Mass., 1957, chapter 3.

(14) M.-M. Janot, R. Goutarel, A. LeHir, G. Tsatsas and V. Prelog, *Helv. Chim. Acta*, **38**, 1073 (1955).

(15) C. F. Huebner, H. B. MacPhillamy, E. Schlittler and A. F. St. André, *Experientia*, **11**, 303 (1955).

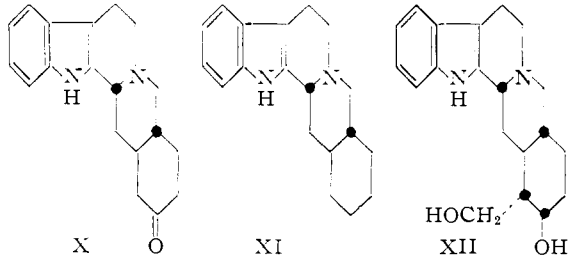
(16) In view of the ease of degradation of the alkaloids of the yohimbine (I) and the corynantheine (III) types to unsubstituted compounds containing merely the asymmetric centers at C-3, 15 and 20 [*cf.* (a) J. E. Saxton, *Quart. Rev.*, **10**, 108 (1956)] and the ready availability of these systems by total synthesis [(b) E. E. van Tamelen, M. Shamma and P. Aldrich, THIS JOURNAL, **78**, 4628 (1956); (c) G. Stork and R. K. Hill, *ibid.*, **79**, 495 (1957); (d) E. E. van Tamelen, P. E. Aldrich and T. J. Katz, *Chemistry & Industry*, 793 (1956)] a general stereochemical method was needed most in the field of alkaloids of the ajmalicine (II) type. No experimental data were available at the start of the present work which could be used in any more than a speculative manner to shed any light on the stereochemistry of substances of the latter class.

(17) While acids can serve as catalysts for C-3 epimerization of any alkaloid under present consideration, acetic anhydride has been useful only in the more activated 11-oxygenated compounds.[13] A recently reported conversion of alloyohimbane to epialloyohimbane by acetic anhydride (A. Chatterjee, S. C. Pakrashi and G. Werner in L. Zechmeister, "Progress in the Chemistry of Natural Products," Vol. XIII, Springer Verlag, Vienna, 1956, p. 357) could not be reproduced in this Laboratory. The exact mechanism of the C-3 equilibration, for which one possible path has been presented already [E. Wenkert and L. H. Liu, *Experientia*, **11**, 302 (1955)], is still under investigation and will be the subject of a future communication.

(18) Whereas this reaction was of interest for its own sake, especially in view of the reported C-3 epimerizations induced by Raney nickel (private communication from Dr. C. F. Huebner) and by platinum [F. L. Weisenborn, THIS JOURNAL, **79**, 4818 (1957)], it was of importance in connection with the mechanism of catalytic hydrogenation of Δ^3-D/E *trans* systems. One of the possible pathways for the latter process (*cf.* footnote 11 in reference 10) consists of the formation of 50% of *normal* and 50% of *pseudo* products and a subsequent platinum-catalyzed isomerization of the latter into the former. Since this path is now excluded, the hydrogenation appears to be a reaction under true thermodynamic control.

(19) W. O. Godfredsen and S. Vandegal, *Acta Chim. Scand.*, **10**, 1414 (1956).

genation of Δ^3-dehydro compounds in the presence of base to a mixture of C-3 epimers was introduced. While the conversion of Δ^3-dehydroyohimbine to a mixture of yohimbine and ψ-yohimbine was readily



X XI XII

duplicable and the yields of products made this reaction a more desirable one than the chemical reduction, it was limited in scope. Δ^3-Dehydrorauwolscine[12] (VII) yielded only rauwolscine (VI) on hydrogenation. Thus it appears that the course of catalytic hydrogenation of didehydro products is governed strictly by steric control, whereas in the absence of any steric factors its course can be altered drastically by slight changes in the reaction medium.

Ring C tetradehydro systems came next under scrutiny. In order to facilitate the study of both their oxidative formation and their reduction, a large number of varied compounds was needed. Since the standard ring C oxidation by lead tetraacetate[16a] was not of general applicability[20] and since it did not lend itself to ready mechanistic interpretation, the palladium–maleic acid method[21] was chosen as the mode of oxidation. While this procedure had lain dormant in the field of indole alkaloids until quite recently,[15,22] it proved to be most versatile, permitting the oxidation of such a wide variety of compounds as: *d,l*-alloyohimbane, yohimbane, yohimbone, yohimbine, ψ-yohimbine, rauwolscine, yohimbyl alcohol, deserpidine, ajmalicine and akuammigine. *Since tetradehydroakuammigine proved to be identical with alstonine,*[16a] *akuammigine is 3-isotetrahydroalstonine.*

Catalytic hydrogenation at pH 10 or sodium borohydride reduction of tetradehydro (IV) systems with D/E trans stereochemistry led exclusively to normal (V) products while those with D/E cis configuration yielded preponderantly allo (VI) products along with traces of epiallo isomers. Reduction of tetradehydroyohimbone nitrate (XIII) gave epiyohimbol, a compound previously obtained by Meerwein–Ponndorf reduction of yohimbone and assigned structure XIV.[3,5,23] Its steric configuration at C-17 was placed on firmer foundation, when it could be shown that lithium aluminum hydride reduction of yohimbone, a process known to lead to equatorial hydroxyl groups,[24] also yielded epiyohimbol (XIV).

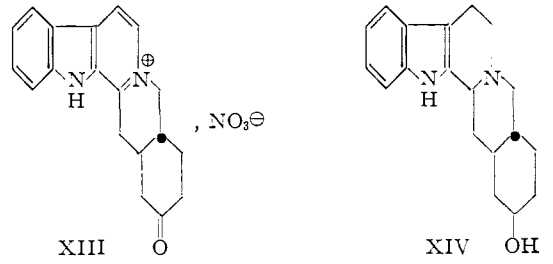
(20) *E.g.*, oxidation of yohimbone by this method has led to no recognizable product.[3]

(21) R. Majima and S. Murahashi, *Proc. Imp. Acad. (Tokyo)*, **10**, 341 (1934) [*C. A.*, **28**, 6720 (1934)]; *Coll. Papers Fac. Sci. Osaka*, **2**, 341 (1935) [*C. A.*, **30**, 3437 (1936)].

(22) Second reference in footnote 17.

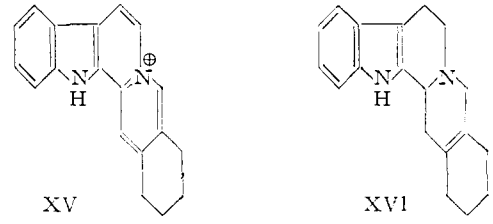
(23) A. Chatterjee, A. K. Bose and S. Pakrashi, *Chemistry & Industry*, 491 (1954).

The ready reductive conversion of serpentine (tetradehydroajmalicine) and alstonine to ajmalicine and tetrahydroalstonine, respectively,[8,16a] suggested strongly that the last two alkaloids belonged to the *normal* (V) or *allo* (VI) class of compounds.



XIII XIV

Sempervirine salts (XV) appeared to be most suitable models for a slight extension of reduction-oxidation studies to systems of higher oxidation state. Catalytic hydrogenation at *p*H 10 had been shown already to yield a mixture of alloyohimbane (VI) and epialloyohimbane (IX).[22] Borohydride reduction, however, gave a yohimbene, which proved to be neither an immonium nor enamine system by the equality of its ultraviolet spectra in neutral or acidic media. Its non-isomerizability by refluxing HBr–HOAc and the identity of its formation with that of a previously investigated N_a-methyl derivative[25] suggested XVI as its structure.[26]



XV XVI

Both palladium–maleic acid and mercuric acetate dehydrogenations of XVI gave 5,6-dihydrosempervirine (XVII, as its salt).[27] The similarity of its ultraviolet spectrum with those of tetrabyrine (XVIII) and alstyrine (XIX)[16a] as well as its ready convertibility to $\Delta^{15(20)}$-yohimbene (XVI) on sodium borohydride reduction and to alloyohimbane (VI) on catalytic hydrogenation at *p*H 10 confirmed its structure. Its production by a mercuric acetate oxidation was reminiscent of the conversion of canadine (XX) to berberine (XXI) by the same reagent.[28]

The availability of a large number of indole alkaloids and their derivatives permitted comparison of their infrared spectra. The region of the stretching vibration of methine C–H groups,[29] 3.5–3.7 μ,

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

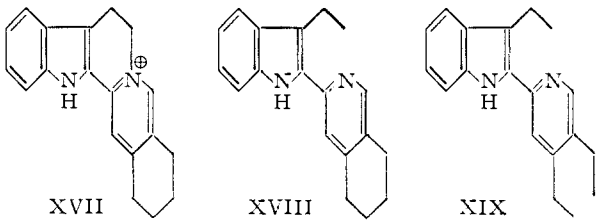
(25) B. Witkop, THIS JOURNAL, **75**, 3361 (1953).

(26) The sodium borohydride reduction of sempervirine (XV) and the acid-catalyzed equilibration of its product (XVI) were first investigated by Dr. L. H. Liu in this Laboratory (unpublished observations).

(27) Dr. C. F. Huebner has kindly informed the authors that in his hands the catalytic dehydrogenation produced sempervirine (XV). Apparently the activity of the catalyst may be critical in determining how far the reaction proceeds.

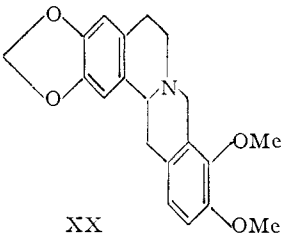
(28) J. Gadamer, *Arch. Pharm.*, **253**, 274 (1915).

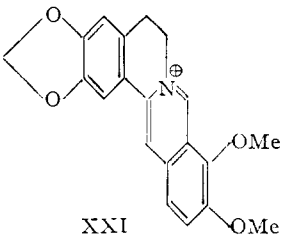
(29) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954.


XVII

XVIII

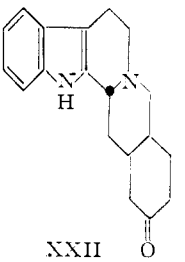
XIX


XX


XXI

proved to be useful for the determination of the C-3 configuration of the alkaloid systems. *Thus, solution or KBr pellet spectra of all compounds containing an α-H at C-3 revealed two or more peaks of medium intensity, while those of β-configuration exhibited merely a shoulder in this region.* This new spectrophotometric method permitted the assignment of stereochemistry for a fair number of alkaloids of unknown configuration.[2a,30] The ajmalicine-like alkaloids (II) of interest for the present investigation could be classified into the following categories. Ajmalicine, tetrahydroalstonine and mayumbine possessed an α-H at C-3, while 3-isoajmalicine and akuammigine had a 3β-configuration.

While being clearly the simplest diagnostic tool for the C-3 configuration, the infrared method remained empirical by nature and as such requires care in application. As first hypothesis it was considered that the infrared data were a reflection of the configuration, possibly conformation, of C-3 and its immediate environment only. The disappearance of any distinction of α- and β-stereochemistry in protic acid salts of the alkaloids was in favor of this suggestion. However, it did not explain the identity of the C–H absorption of epialloyohimbane and 3-epi-α-yohimbine despite the difference of conformation of their C-3 hydrogen atoms.[22] Finally, it was incompatible with the absence of any shift in the C–H region on the introduction of deuterium at C-3.[31] It thus appears that the infrared effect must be due to the vibrational interaction of several methine linkages of which C-3, 15 and 20 may be most important. The difference of C–H absorption of yohimbane and $\Delta^{15(20)}$-yohimbene strongly supports this view. So does the noncorrelatibility of the spectra of reserpine and 3-isoreserpine lactones, the conformation of whose bridgehead hydrogen atoms cannot be depicted with certainty, with those of their parent compounds. Finally, the latter interpretation of the spectroscopic evidence would suggest that the infrared spectrum of epialloyohimbone (XXII), a compound of ambiguous conformation because of the presence of both a carbonyl group and a D/E *cis* ring juncture, would be unpredictable.[32] Indeed it proved to be an exception to the uniformity of data, since its spectrum made it resemble a compound with α-C-3 configuration.


XXII

Until now all accumulated data had revolved mostly around the stereochemistry of C-3 but had revealed only little about C-15 and 20. Access to these centers seemed to lie in any method of differentiation between D/E *cis* and *trans* systems. One such procedure had been reported in connection with controlled high temperature catalytic dehydrogenation of alkaloid derivatives. Yohimbane (V) had yielded its tetradehydro derivative (IV), while alloyohimbane (VI) had led to sempervirine (XV).[33] Unfortunately the generality of this method was in question because of the lack of distinction between the *cis*- and *trans*-17,18-seco compounds XXIII,[16d] both of them leading to the octadehydro product XXIV.[34] When, nevertheless, ajmalicine (II) was exposed to the same dehydrogenation scheme, it was transformed into a desoxygenated material of less carbon content.[35] Hence this procedure was of no stereochemical aid for alkaloids of structure II.

Finally, advantage was taken of the known dif-

(30) Raumitorine and reserpiline were two compounds whose assignment was only tentative because of the uncertainty of their spectra (*cf.* ref. 1a, footnote 16). A clearer spectrum of the former, presumably of a purer sample, shows that raumitorine has an α-H configuration at C-3.

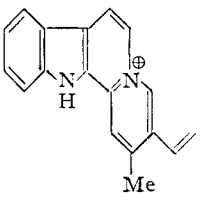
(31) Unpublished observation of This Laboratory.

(32) As in A/B *cis* 3-keto steroids [*cf.* H. R. Nace and R. B. Turner, THIS JOURNAL, **75**, 4063 (1953)] the oxygenated ring may exist partly in a boat form. However, unlike the former cases this conformational change from the norm may be transmitted through the entire conformationally flexible ring system in epialloyohimbone in such a way as to make the assignment of the most stable conformer quite insecure. One further factor obscuring the picture is the possibility of a favorable electronic interaction between Nb and C-17, making the following di-boat form a conformational example not lightly dismissed.

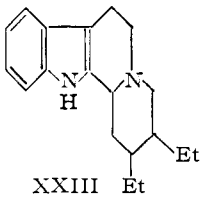
(33) A. LeHir, R. Goutarel and M.-M. Janot, *Bull. soc. chim., France*, 866 (1954). Repetition of this work by Dr. L. H. Liu (unpublished observations) led to identical results.

(34) R. Goutarel, M.-M. Janot and M. C. Perezmador y Barron, *ibid.*, 863 (1954).

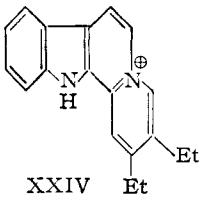
(35) Its elementary analysis, its lack of optical activity, the striking similarity of its ultraviolet spectrum to that of sempervirine and a rational mechanistic interpretation of its mode of formation permit the assignment of the following tentative formula for its salts.


Me

The identical compound, compared by mixed melting point, has been obtained recently by Professor Uyeo by a palladium dehydrogenation of serpentine in xylene solution. The authors wish to express their thanks for this information and for a comparison of the two compounds in the Osaka Laboratory. An exact structure proof of the degradation product as well as a study of the general utility of the high temperature dehydrogenation process is now under investigation.

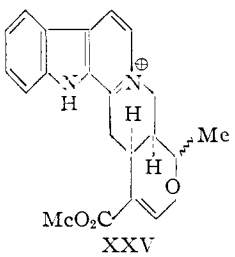


XXIII

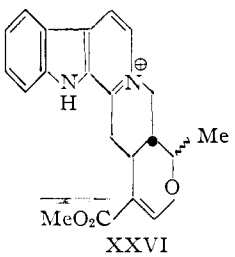


XXIV

ference of the rate of palladium-maleic acid dehydrogenation of *epiallo* and other systems.[22] A series of simultaneous, identical dehydrogenations were carried out on various sets of two to four compounds of identical functionality but different stereochemistry. The reactions were arbitrarily quenched early and the ultraviolet spectra of the solutions compared with those of standardized mixtures of yohimbine and tetradehydroyohimbine perchlorate. The resultant qualitative data clearly pointed up the fact that *epiallo* (IX) compounds, whose oxidizable hydrogen atom at C-3 is the least exposed toward access of a catalytic surface as compared to the same site in stereochemically different systems, undergoes catalytic oxidation slower than compounds of other configurations. Thus this method appeared to be capable of differentiating between diastereomers of C-3 β-configuration (VIII and IX), *e.g.*, 3-isoajmalicine and akuammigine. Identical oxidations, with early arbitrary quenching, caused these compounds to yield 0 and 90% tetradehydro products, respectively. Consequently, the former appears to be an *epiallo* compound and the latter a *pseudo* product. Furthermore, structures XXV and XXVI can now be assigned to the salts of serpentine and alstonine, respectively.[36,37]



XXV



XXVI

Acknowledgment.—The authors express their sincere gratitude to Sir Robert Robinson, Professor Janot and Drs. Aghoramurthy, Diassi, Hofmann, Klohs and Neuss for a generous supply of alkaloids. They also feel greatly indebted to Ciba Pharmaceutical Products, Inc., and especially to Drs. Huebner, Lucas, MacPhillamy, Schlittler and Ulshafer for the many alkaloids and derivatives they supplied and the stimulating discussions in which they engaged. Many thanks to the Institute for Atomic Research, Ames, Iowa, for the use of a Baird infrared spectrophotometer, and to the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare, for a generous research grant (M 1301) in support of part of the present work.

(36) Identical stereochemical conclusions obtained by different means have been reported recently by N. Neuss and H. E. Boaz [*J. Org. Chem.*, **22**, 1001 (1957)].

(37) The dehydrogenation procedure appears to be of general stereochemical applicability for ring A unoxygenated alkaloids. Ring A methoxylated systems of 3-β-orientation unfortunately would epimerize at C-3 in the maleic acid medium too rapidly to yield unambiguous results. For this reason a direct interconnection between alkaloids with oxygen in ring A and those without is necessary. Such interconversion should be possible by the catalytic hydrogenation in acid medium of ring A of tetradehydro derivatives of both types of compounds (*cf.* H. Schwarz and E. Schlittler, *Helv. Chim. Acta*, **34**, 629 (1951)). The recent conversion of reserpine and deserpidine to the same tetrahydro-tetradehydro derivative (E. Wenkert, E. W. Robb and N. V. Bringi, THIS JOURNAL, **79**, 6570 (1957)) serves as example of he power of this method.

Experimental

Equilibration of Pseudoyohimbine. (a).—A solution of 200 mg. of pseudoyohimbine, 5 ml. of 48% hydrobromic acid and 5 ml. of glacial acetic acid was refluxed for three hours. The solvent was removed under vacuum, the residue dissolved in methanol and treated with an excess of ethereal diazomethane solution. After standing for ten minutes, the solution was evaporated to dryness and the residue chromatographed on alumina. Chloroform elution yielded an oil which crystallized in ethanol, yielding 34 mg. of yohimbine, as shown by m.p. 236–237°, mixed m.p. and infrared spectrum.

(b).—A mixture of 50 mg. of pseudoyohimbine, 15 mg. of platinum oxide in 25 ml. of methanol was shaken under hydrogen at 57 lb./sq. in. for three hours. The solution then was filtered, the solvent removed and the residue crystallized from ethanol. Pseudoyohimbine (30 mg.), identified by m.p. 272–273°, mixed m.p. and infrared spectrum, was recovered.

Mercuric Acetate Oxidation of Yohimbone and Epialloyohimbone.—When yohimbone was oxidized in a manner previously described,[10] a 54% yield of yellow granules of 3-dehydroyohimbone perchlorate, m.p. 181–182°, $[\alpha]_D$ +114° (methanol), was obtained.

Anal. Calcd. for $C_{19}H_{21}O_5N_2Cl \cdot 2CH_3OH$: C, 55.17; H, 6.39; N, 6.13. Found: C, 54.84; H, 6.55; N, 5.82.

Oxidation of epialloyohimbone yielded no mercurous acetate even after eight hours. The ultraviolet spectrum of the crude mercury-free perchlorate showed no appreciable dehydrogenation.

Synthesis of Pseudo Compounds. (a).—A suspension of the 3-dehydro perchlorate and a large excess of zinc dust, five-to-six times the weight of the 3-dehydro compound, in glacial acetic acid was refluxed for two hours. The mixture was filtered, the solvent removed under vacuum, the residue dissolved in aqueous methanol and made basic with concentrated ammonia. The precipitated base was extracted exhaustively with chloroform, the extract washed, dried and evaporated. The residue was chromatographed on alumina.

Chloroform elution gave an 11% yield of pseudoyohimbine, m.p. 277–278°, and 7% yohimbine, m.p. 234–235°, identified by mixed m.p. and infrared spectra.

Benzene–ether (1:1) elution led to a 6% yield of yohimbane, m.p. 204–205°, identified by mixed m.p. and infrared spectrum and 11% pseudoyohimbane, m.p. 95–96° $[\alpha]_D$ +62° (ethanol).

Anal. Calcd. for $C_{19}H_{24}N_2$: C, 81.37; H, 8.63; N, 9.99. Found: C, 81.32; H, 8.57; N, 9.59.

Elution with 7:3 and 3:7 benzene–ether produced 13% yohimbone, m.p. 305–306°, and 7% pseudoyohimbone, m.p. 270–273°, $[\alpha]_D$ −24° (pyridine), respectively, as identified by m.p., infrared spectra and optical rotation.

Benzene and 4:1 benzene–ether elution gave 18% 3-isoajmalicine, m.p. 193–194°, $[\alpha]_D$ −122° (pyridine) (*Anal.* Calcd. for $C_{21}H_{24}O_3N_2$: C, 71.55; H, 6.86; N, 7.95. Found: C, 71.39; H, 6.96; N, 7.79) and 13% ajmalicine, m.p. 256–257°, respectively, identified by mixed m.p. and infrared spectra.

(b).—A mixture of 150 mg. of lithium aluminum hydride in 10 ml. of purified tetrahydrofuran, to which there had been added 300 mg. of pseudoyohimbine in 10 ml. of ether and 10 ml. of tetrahydrofuran, was refluxed gently for six hours. After cooling and a drop-by-drop addition of saturated aqueous sodium sulfate solution, the mixture was filtered and the filtrate evaporated under vacuum. Chromatography of the residue on alumina and elution with chloroform containing 5% methanol yielded an oil which failed to crystallize. Dissolution thereof in 5% aqueous acetic acid and addition of 70% perchloric acid gave a precipitate which on crystallization from methanol yielded 165 mg. (46%) of pseudoyohimbyl alcohol perchlorate, m.p. 290–291°. Comparison with yohimbyl alcohol perchlorate,

m.p. 280–281°, by mixed m.p. and infrared spectra proved them to be different.

Anal. Calcd. for $C_{20}H_{27}O_6N_2Cl$: C, 56.27; H, 6.38; N, 6.56. Found: C, 56.33; H, 6.49; N, 6.46.

Hydrogenation of Δ^3-Dehydrorauwolscine Perchlorate.—A mixture of 285 mg. of Δ^3-dehydrorauwolscine, 40 mg. of platinum oxide and 3 ml. of triethylamine in 20 ml. of methanol was hydrogenated under 50 lb./sq. in. of hydrogen pressure for six hours. After filtration and concentration of the mixture, the residue was dissolved in aqueous methanol, made basic with concentrated ammonia and extracted with chloroform. The extract was washed with aqueous saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated. Chromatography of the residue on alumina and elution with chloroform gave a fraction which on crystallization in methanol yielded 100 mg. (45%) of rauwolscine, m.p. 235–237°, identical in m.p. and infrared spectrum with authentic material.

Dehydrogenations with Palladium and Maleic Acid.—In a typical run, 1 mmole of the amine and 5 mmoles of maleic acid were dissolved in water and palladium black equal in weight to 0.5 mmole of the amine was added. The mixture was refluxed for eight hours and then filtered while hot. On cooling and addition of 70% aqueous perchloric acid, a tetradehydro perchlorate was obtained. In some cases the base was precipitated initially by the addition of concentrated ammonia, and dissolution of the free base in 5% aqueous acetic acid, followed by addition of aqueous ammonium nitrate or potassium perchlorate solutions, led to the tetradehydro salt. The precipitated salt was filtered and crystallized from methanol.

d,l-Alloyohimbane gave a 49% yield of yellow needles of *d,l*-tetradehydroalloyohimbane perchlorate, m.p. 207–208°; ultraviolet spectrum (ethanol): λ_{max} 253 mμ (log ε 4.31), 307 mμ (log ε 4.15) and 367 mμ (log ε 3.61); λ_{min} 228 mμ (log ε 4.01), 278 mμ (log ε 3.58) and 325 mμ (log ε 3.30). *Anal.* Calcd. for $C_{19}H_{21}O_4N_2Cl$: C, 60.54; H, 5.58; N, 7.44. Found: C, 60.54; H, 5.83; N, 7.34.

Yohimbane yielded yellow needles of tetradehydroyohimbane nitrate (51%), m.p. 259–260°, $[\alpha]_D$ +120° (methanol). *Anal.* Calcd. for $C_{19}H_{21}O_3N_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.51; H, 6.37; N, 12.14.

Yohimbone led to a 63% yield of rosy granules of tetradehydroyohimbone nitrate, m.p. 275–277°, $[\alpha]_D$ +92.6° (methanol). *Anal.* Calcd. for $C_{19}H_{19}O_4N_3 \cdot CH_3OH$: C, 62.32; H, 6.01; N, 10.90. Found: C, 62.41; H, 5.91; N, 10.96.

Yohimbine and pseudoyohimbine gave tetradehydroyohimbine perchlorate, colorless needles, m.p. 200–201°, $[\alpha]$ +181° (methanol), in 74 and 64% yields, respectively. *Anal.* Calcd. for $C_{21}H_{23}O_7N_2Cl \cdot CH_3OH$: C, 54.71; H, 5.64; N, 5.80. Found: C, 54.64; H, 5.30; N, 5.96.

Rauwolscine yielded cream-colored needles of tetradehydrorauwolscine perchlorate (44%), m.p. 250–251°, $[\alpha]_D$ +99.4° (methanol). *Anal.* Calcd. for $C_{21}H_{23}O_7N_2Cl \cdot CH_3OH$: C, 54.71; H, 5.64. Found: C, 54.4; H, 5.45.

Yohimbyl alcohol gave cream-colored needles of tetradehydroyohimbyl alcohol perchlorate (40%), m.p. 250–251°, $[\alpha]_D$ +145.4° (methanol). *Anal.* Calcd. for $C_{20}H_{23}O_6NCl \cdot CH_3OH$: C, 55.43; H, 5.98; N, 6.16. Found: C, 55.32; H, 5.92; N, 6.54.

Deserpidine was converted to tetradehydrodeserpidine perchlorate (54%), yellow-green needles, m.p. 190–192°, $[\alpha]_D$ −189° (methanol). *Anal.* Calcd. for $C_{32}H_{35}O_{12}N_2Cl$: C, 56.92; H, 5.23; N, 4.15. Found: C, 56.66; H, 5.67; N, 4.27.

Ajmalicine yielded serpentine nitrate (43%), m.p. 161–163°, as identified by mixed m.p. and ultraviolet and infrared spectra.

Akuammigine yielded alstonine perchlorate (42%), m.p. 247–248°, $[\alpha]_D$ +152° (methanol), as shown by comparison of its melting point, rotation and infrared spectrum with authentic material.

Reduction of Tetradehydro Compounds. (a) Catalytic Hydrogenation.—A mixture of 3 mmoles of tetradehydro nitrate or perchlorate in 60 ml. of methanol, 2 ml. of 2 *N* methanolic potassium hydroxide and 250 mg. of platinum oxide was hydrogenated at 57 lb./sq. in. for 12 hours. The solution was filtered, the filtrate neutralized with 2 *N* methanolic hydrochloric acid, the solvent evaporated, the residue dissolved in water and the base precipitated with concentrated ammonia. The precipitate was filtered, washed free of ammonia and crystallized in methanol. The crystalline bases were identified by melting point, mixed melting point and infrared spectra.

Tetradehydroyohimbane nitrate gave 48% yohimbane, m.p. 204–205°.

d,l-Tetradehydroalloyohimbane perchlorate gave 54% *d,l*-alloyohimbane, m.p. 147–148°. Evaporation of the crystallization mother liquor, chromatography on alumina and 1:1 petroleum ether–ether elution yielded 0.4% *d,l*-epialloyohimbane, m.p. 194–195°, no depression on admixture with an authentic sample.

Serpentine nitrate produced 63% ajmalicine, m.p. 249–250°.

Tetradehydroyohimbone nitrate gave 75% epiyohimbol (XIV), m.p. 257-258°, no depression when admixed with authentic sample (*vide infra*).

(b) **Sodium Borohydride Reduction.**—A solution of 0.8 mmole of the tetradehydro compound and 500 mg. (13 mmoles) of sodium borohydride in 30 ml. of methanol was refluxed for 2 hours. The solvent was evaporated under vacuum, the residue treated with 10 ml. of water and the suspension extracted with chloroform. The extract was washed, dried over anhydrous sodium sulfate, evaporated and the residue crystallized in methanol. The following bases, characterized by their m.p., mixed m.p. and infrared spectra, were obtained: 38% yohimbane, m.p. 204–205°; 58% *d,l*-alloyohimbane, m.p. 145–146°; 20% yohimbine, m.p. 230–231°; 47% epiyohimbol (XIV), m.p. 256–257°; 50% ajmalicine, m.p. 253–254°.

Epiyohimbol (XIV).—A solution of 500 mg. of yohimbone in 50 ml. of tetrahydrofuran was added dropwise to a slurry of 200 mg. of lithium aluminum hydride in 25 ml. of tetrahydrofuran, and the mixture refluxed for 12 hours. After cooling, the reaction mixture was decomposed by a dropwise addition of saturated aqueous sodium sulfate solution. The solid mass was washed with ether, the combined ether-tetrahydrofuran solution dried over anhydrous sodium sulfate, the solvent evaporated and the residue crystallized in methanol, yielding 75 mg. (15%) of epiyohimbol, m.p. 262–263°, as fine colorless needles. An additional 125 mg. (25%) of epiyohimbol, m.p. 254–255°, identified by mixed m.p. and infrared spectrum, was obtained from the mother liquor.

***d,l*-$\Delta^{15(20)}$-Yohimbene (XVI).**—A mixture of 1.76 g. (5.3 mmoles) of sempervirine nitrate and 1.5 g. (39 mmoles) of sodium borohydride in 20 ml. of methanol was refluxed for two hours. After removal of the solvent under vacuum and trituration of the residue with water, the suspension was extracted with chloroform, the extract dried over anhydrous sodium sulfate and the solvent evaporated. The resultant yellow-brown residue was chromatographed on alumina as a 1:1 petroleum ether–ether solution and eluted with the same pair, yielding 700 mg. (43%) of *d,l*-$\Delta^{15(20)}$-yohimbene, m.p. 190°. Three crystallizations from methanol gave colorless needles, m.p. 196–197°; ultraviolet spectrum: λ_{max} 225 mμ (log ε 4.55), 282 mμ (log ε 3.89) and 290 mμ (log ε 3.80); λ_{min} 250 mμ (log ε 3.38).

Anal. Calcd. for $C_{19}H_{22}N_2$: C, 81.97; H, 7.97; N, 10.06. Found: C, 81.57; H, 7.72; N, 9.93.

5,6-Dihydrosempervirine Perchlorate (XVII).—A mixture of 200 mg. of *d,l*-$\Delta^{15(20)}$-yohimbene (XVI), 400 mg. of maleic acid and 100 mg. of palladium black in 25 ml. of water was refluxed and stirred for ten hours. The mixture was filtered while hot and the orange-yellow filtrate made basic with concentrated ammonia. The precipitated base was filtered and dissolved in 20 ml. of 5% aqueous acetic acid. Addition of saturated aqueous ammonium nitrate solution led to 100 mg. (41%) of crude 5,6-dihydrosempervirine nitrate (XVII). Four crystallizations of the compound in methanol yielded red crystals, m.p. 305–306°; ultraviolet spectrum: λ_{max} 223 mμ (log ε 4.58) and 320 mμ (log ε 4.33); λ_{min} 276 mμ (log ε 3.85).

Anal. Calcd. for $C_{19}H_{19}O_3N_3$: C, 67.64; H, 5.68; N, 12.46. Found: C, 68.06; H, 5.60; N, 12.27.

A solution of 65 mg. (0.23 mmoles) of *d,l*-$\Delta^{15(20)}$-yohimbene (XVI) and 50 mg. (1.57 mmoles) of mercuric acetate in 15 ml. of 5% aqueous acetic acid was heated under nitrogen at 60° for four hours. Filtration of the cooled reaction mixture yielded 70 mg. (58%) of mercurous acetate. The filtrate was heated to boiling, hydrogen sulfide gas introduced, the insoluble sulfides filtered, and a saturated aqueous potassium perchlorate solution added. The resultant

precipitate gave 17 mg. (20%) of orange-red crystals of 5,6-dihydrosempervirine perchlorate (XVII), m.p. 308–309°, on crystallization in methanol.

Dissolution of the perchlorate in a small amount of methanol and 5% aqueous acetic acid and addition of saturated aqueous ammonium nitrate solution led to the nitrate. Several crystallizations in methanol yielded red needles of 5,6-dihydrosempervirine nitrate (XVII), m.p. 305–306°, identical in m.p., mixed m.p. and ultraviolet and infrared spectra with a sample from the catalytic dehydrogenation reaction.

Reduction of 5,6-Dihydrosempervirine Nitrate (XVII).—When a catalytic hydrogenation was carried out on 410 mg. of XVII by the method described above, 135 mg. (40%) of crude alloyohimbane was obtained. Recrystallization in methanol yielded crystalline *d,l*-alloyohimbane, m.p. 144–145°, identical in m.p., mixed m.p. and infrared spectrum with an authentic specimen.

When a sodium borohydride reduction was carried out on 210 mg. of XVII by the method described above, 65 mg. of pure *d,l*-$\Delta^{15(20)}$-yohimbene (XVI), m.p. 194–195°, identified by mixed m.p. and infrared spectrum, was obtained.

Qualitative Data of the Rate of Catalytic Dehydrogenation.—Aqueous fumaric acid solutions (0.44%) of 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 yohimbine–tetradehydroyohimbine perchlorate mixtures were prepared and their ultraviolet spectra (220–370 mμ) determined. A graph of the resulting six curves was used as standard for determining the contents of dehydrogenation mixtures.

In a typical run, used to diagnose the extent of dehydrogenation, 0.05 mole of the amine was dissolved in 8 ml. of 0.44% aqueous maleic acid solution, 15 mg. of palladium black added and the mixture stirred and refluxed. After filtration the solution was diluted to 10 ml. with water and a 0.4-ml. aliquot diluted to 100 ml. The ultraviolet spectra were plotted and the curves compared with standards. The intensity of the 248, 305 and 365 mμ peaks determined the degree of dehydrogenation.

Four-hour runs with one fairly active batch of palladium black led to the following results: I, (1) yohimbine 95% dehydrogenated, (2) pseudoyohimbine 95%, (3) rauwolscine 95% and (4) 3-epi-α-yohimbine 20%; II, (1) pseudoyohimbyl alcohol 90% and (2) 3-epi-α-yohimbyl alcohol 70%; III (1) pseudoyohimbane 50% and (2) *d,l*-epialloyohimbane 45%. Eight-hour runs with less active catalyst: I, (1) yohimbine 90%, (2) pseudoyohimbine 90%, (3) rauwolscine 90% and (4) 3-epi-α-yohimbine 80%; II, (1) pseudoyohimbyl alcohol 90% and (2) 3-epi-α-yohimbyl alcohol 65%; III, (1) apoyohimbine 80%, (2) aporauwolscine 80%, (3) apo-3-epi-α-yohimbine 30% and (4) *d,l*-epialloyohimbane 40%; IV, (1) ajmalicine 80%, (2) 3-isoajmalicine 0% and (3) akuammigine 90%.

AMES, IOWA

[CONTRIBUTION FROM THE DIVISIONS OF PHARMACOLOGY AND BIOCHEMISTRY, DEPARTMENT OF RADIATION BIOLOGY, SCHOOL OF MEDICINE AND DENTISTRY, THE UNIVERSITY OF ROCHESTER]

Concerning the Mechanism of Action of Parathyroid Hormone I Ion-Gradients[1]

BY H. FIRSCHEIN, G. MARTIN, B. J. MULRYAN, B. STRATES AND W. F. NEUMAN

RECEIVED OCTOBER 11, 1957

Citrate ion has been shown to exert a powerful solubilizing action on hydroxy apatite and on bone mineral. A net production of citrate by bone and its transfer to the circulation has been demonstrated by arteriovenous differences coupled with radiostrontium clearances. The general effects of parathyroid activity on citrate metabolism has been confirmed. Following intravenous administration of parathyroid extracts, increased citrate levels (also increased phosphate levels, by exchange-displacement with citrate?) in serum *preceded* the rise in serum calcium. In keeping with recent data on the solubility of bone, a general hypothesis of the possible mechanism of the action of the parathyroid secretions on bone is given.

Ever since Dickens first demonstrated that bone contains relatively large quantities of citrate,[2,3] there has been a continuing interest in the possibility that this organic anion is of importance in calcium metabolism. Two recent reviews document this interest[4,5] which has prompted numerous suggestions concerning the role of citrate in the homeostatic regulation of calcium levels in serum by the actions of vitamin D and of parathyroid secretions. Reported here are a series of studies designed to clarify the importance of citrate metabolism in the mediation of the action of the parathyroid secretions on bone.

Confirmation of the Correlation between Parathyroid Activity and Serum Citrate Levels.—Mongrel dogs, six months old, were used in this experiment. Three animals were thyroparathyroidectomized, under Nembutal anesthesia, and then maintained for three days on lean hamburger and water *ad libitum*. Two animals served as controls (no sham operation) while two additional dogs received subcutaneous injections of parathyroid extract (Eli Lilly Co.) as follows: 200 units 48 hr., 100 units 36 hr. and 500 units 24 hr. prior to sacrifice. All animals were anesthetized with Nembutal for the withdrawal of samples of venous blood from the jugular vein. Serum was analyzed for calcium[6] and citrate.[7] In addition, the serum samples were ultrafiltered[8,9] to estimate the levels of diffusible calcium, citrate and phosphate.[10] These results are summarized in Table I.

Grossly, these data show that parathyroidectomy decreases the amount of circulating citrate, while injections of parathyroid extracts increase serum citrate. This correlation between serum citrate

(1) This paper is based in part on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York, and in part on work supported by a grant, A1209, from the National Institutes of Arthritis and Metabolic Diseases, U. S. Public Health Service. The authors gratefully acknowledge the assistance of V.DiStefano and D. Leary in developing the surgical procedures.

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