Revised Structures of the *Pleiocarpa* Alkaloids Pleiocarpoline (Pleiocarpine N_b-Oxide), Pleiocarpolinine (Pleiocarpinine N_b-Oxide), and Kopsinoline (Kopsinine N_b-Oxide)

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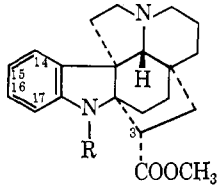
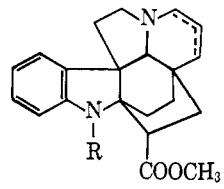
Abstract: Three alkaloids were isolated from the extract of *Pleiocarpa mutica* Benth. On the basis of their mass and nmr spectra as well as chemical behavior, they were assigned structures VIII, IX, and X, the N_b-oxides of pleiocarpine, pleiocarpinine, and kopsinine. Partial synthesis of VIII by oxidation of pleiocarpine confirmed the correctness of this assignment. The same alkaloids had been isolated very recently by Kump, Seibl, and Schmid and suggested to be 3-hydroxymethyl derivatives (XI, XII, and XIII) of pleiocarpine, pleiocarpinine, and kopsinine. The arguments which led the Swiss group to this assignment are discussed in the light of our structures.

In continuation of our work[1] on the alkaloids of *Pleiocarpa mutica* Benth, three new alkaloids, A, B, and C, were obtained by repeated chromatography of the crude stem bark extract on both alumina and silicic acid.

Mass spectra showed that the three alkaloids were closely related; however, although the compounds were introduced directly into the ion source of the spectrometer, each spectrum was the result of thermal decomposition of the sample, which produced a mixture of at least two products. For example, compound A gave a mass spectrum with "molecular ions" at *m/e* 396 and 394. Accompanying peaks at *m/e* 109, 124, and 368 (M − 28) suggested that one of these products was pleiocarpine (I),[2,3] whereas small peaks at *m/e* 107 and 122 indicated a dehydrogenated pleiocarpine (mol wt 394). The latter may be represented by structure IV, since the ions of *m/e* 109 and 124 of pleiocarpine (which contain the piperidine ring[2,4]) are each found two mass units lower. Likewise, the spectra of B and C consisted mainly of peaks from pleiocarpinine (II)[1d,2] and kopsinine (III),[2] respectively, together with their dehydrogenated derivatives, V and VI.



I, R = $COOCH_3$
II, R = CH_3
III, R = H



IV, R = $COOCH_3$
V, R = CH_3
VI, R = H

Ultraviolet spectra of A, B, and C were identical with those[5] of pleiocarpine, pleiocarpinine, and kopsinine, respectively, which implied that the new alkaloids contained an indoline chromophore differing only in substitution at the nitrogen. Infrared and nmr spectra further confirmed the N_a substituents to be $COOCH_3$, CH_3, and H, respectively.

Because of the obvious relationship of the three alkaloids, experiments aimed at the elucidation of their structure were mainly performed on the N_a-carbomethoxy derivative, A, and the N_a-methyl derivative, B.

Upon catalytic reduction alkaloid A was converted in high yield to a single product which was identified (mixture melting point, mass and infrared spectra, rotation, and R_f) as pleiocarpine (I), a result which further confirmed the close structural relationship of these two alkaloids. Similarly, B and C were converted to pleiocarpinine (II) and kopsinine (III), as indicated from the mass spectra and R_f values of the reduction products.

A result, very surprising at first, was the conversion of B by reduction with lithium aluminum hydride to N_a-methylkopsinyl alcohol (VII). This requires that the functionality producing the difference between the pleiocarpine series and the new compounds is removed by catalytic as well as hydride reduction. Furthermore, reduction of alkaloid B with lithium aluminum deuteride gives the same product (VII-d_2) as is obtained from II, thus excluding a reducible group that would lead to a new C–H bond.

Of all the chemical and physical characteristics of these alkaloids, only two are of importance and deserve detailed discussion.

First, the nmr spectra of these alkaloids (Figure 1) all exhibit a signal near 8 ppm (double doublet, J = 7, 2 cps, corresponding to one hydrogen) not present in the spectra of I, II, and III. Secondly, they are much more polar (very low R_f on tlc, soluble in water!) than indole alkaloids usually are.

From a consideration of the nmr spectra of all three alkaloids, the above mentioned signal downfield from the aromatic region must be due to the hydrogen at C-14. The hydrogen at C-17 is ruled out because this is visible as the broad doublet near 7.5 ppm in the spectra

(1) (a) H. Achenbach and K. Biemann, *Tetrahedron Letters*, 3239 (1965); (b) H. Achenbach and K. Biemann, *J. Am. Chem. Soc.*, **87**, 4177 (1965); (c) H. Achenbach and K. Biemann, *ibid.*, **87**, 4944 (1965); (d) D. W. Thomas, H. Achenbach, and K. Biemann, *ibid.*, **88**, 1537 (1966).

(2) W. G. Kump, D. J. LeCount, A. R. Battersby, and H. Schmid, *Helv. Chim. Acta*, **45**, 854 (1962).

(3) C. Djerassi, T. George, N. Finch, H. F. Lodish, H. Budzikiewicz, and B. Gilbert, *J. Am. Chem. Soc.*, **84**, 1499 (1962).

(4) C. Djerassi, H. Budzikiewicz, R. J. Owellen, J. M. Wilson, W. G. Kump, D. J. LeCount, A. R. Battersby, and H. Schmid, *Helv. Chim. Acta*, **46**, 742 (1963).

(5) N. Neuss, "Physical Data of Indole and Dihydroindole Alkaloids," Vol. II, Eli Lilly & Co., Indianapolis, Ind., 1962.

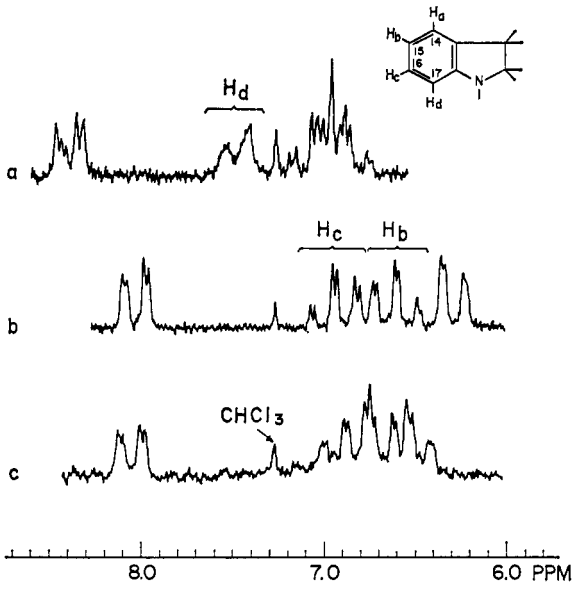


Figure 1. Partial nmr spectra of (a) alkaloid A (VIII), (b) alkaloid B (IX), and (c) alkaloid C (X).

of both A (Figure 1a) and I (shifted due to the carbomethoxy group at N_a). The hydrogens at C-15 and C-16 can be most definitely identified in the spectrum of B (Figure 1b) as a six-line pattern for each, at 6.60 and 6.93 ppm.

This large downfield shift of the hydrogen attached to C-14 requires a change in electron distribution in the vicinity of this carbon atom, and since this functionality cannot be located at a neighboring carbon atom, it must be a group that is rigidly held near this location. Inspection of a Dreiding model of pleiocarpine (I) reveals that N_b is relatively close to the upper part of the benzene ring and that an appropriate modification of the tertiary nitrogen atom or its vicinity could well give rise to this remarkable shift of the hydrogen at C-14.

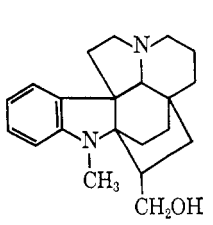
The most plausible group that could give rise to this shift and would, at the same time, satisfy all other properties of these new alkaloids (polarity, conversion to the pleiocarpine series by *both* catalytic and hydride reduction) is an N_b-oxide.

The conversion of the new alkaloids to the pleiocarpine series by reducing agents is in agreement with expectations and a similar conversion upon pyrolysis in the mass spectrometer as well as in a tube (see below) is also not surprising. The mass spectra of some simple, aromatic N-oxides have been determined.[6] All showed a strong $(M - 16)^+$ ion, which may have been formed thermally, since when a heated inlet system was used only the spectra of the corresponding amines were obtained. Furthermore, venoxidine, an indole N-oxide, also shows no molecular ion but only the spectrum of the amine.[7] The appearance of a dehydropleiocarpine in the mass spectrum of alkaloid A (and the analogous behavior of B and C) is explained by a rearrangement of the N-oxide to an amino alcohol which then loses the elements of water.

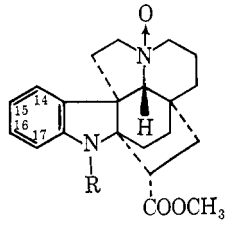
(6) T. A. Bryce and J. R. Maxwell, *Chem. Commun.* (London), 206 (1965).

(7) A. Chatterjee, P. L. Majumder, and A. B. Ray, *Tetrahedron Letters*, 159 (1965).

The presence of an N-oxide was confirmed by reduction of alkaloid A to pleiocarpine in high yield using ferrous sulfate, a reagent considered to be rather specific for N-oxides.[7-9] The three alkaloids were thus assigned structures of pleiocarpine N_b-oxide (VIII) for A, pleiocarpinine N_b-oxide (IX) for B, and kopsinine N_b-oxide (X) for C.

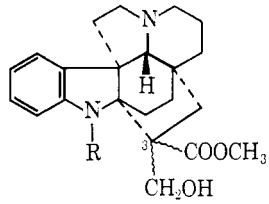


VII



VIII (A), R = $COOCH_3$
IX (B), R = CH_3
X (C), R = H

At about that time there appeared a paper by Kump, Seibl, and Schmid[10] in which they discussed three new alkaloids, pleiocarpoline, pleiocarpolinine, and kopsinoline, isolated from *Hunteria eburnea* Pichon, *Pleiocarpa pycnantha* (K. Schum.) Stapf *var. tubicina* (Stapf) Pichon, and *P. mutica* Benth. These alkaloids were assigned the structures of 3-hydroxymethylpleiocarpine (XI), -pleiocarpinine (XII), and -kopsinine (XIII). From the physical data and chemical behavior



XI, R = $COOCH_3$
XII, R = CH_3
XIII, R = H

reported there was no doubt that their three alkaloids were identical with ours,[11] and it remained to resolve the discrepancy in the structures proposed.

It was felt that a partial synthesis of the natural products would be the most conclusive evidence, and for obvious reasons the N_b-oxide VIII was prepared by oxidation[12] of pleiocarpine with hydrogen peroxide in ethanol. It was identical in every respect with our alkaloid A (peliocarpoline), thus unambiguously confirming structure VIII rather than XI for this alkaloid. Similar oxidation of pleiocarpinine led to no chloroform-extractable products, presumably because of

(8) W. R. Dunstan and E. Goulding, *Trans. Chem. Soc.*, **75**, 792, 1004 (1899).

(9) C. C. J. Culvenor, *Rev. Pure Appl. Chem.*, **3**, 83 (1953).

(10) C. Kump, J. Seibl, and H. Schmid, *Helv. Chim. Acta*, **48**, 1002 (1965).

(11) The identity of these compounds is based primarily on the following facts: they were obtained from the same plant species; they exhibit the same unusual chemical behavior; optical rotations were identical; and all compounds gave the unusual nmr signal near 8 ppm. This last feature alone is strong evidence for the identity of our compounds, since this absorption is well downfield from normal indoline absorption and could not be convincingly accounted for by the hydroxymethylene structures. Although the higher melting points of our compounds could indicate a higher degree of purity, the comparison of the values obtained under nonequivalent conditions for thermally labile compounds does not seem to be a reliable criterion for their identity. The infrared and nmr data differ slightly in individual peak positions but always in the same direction, as might be expected from spectra determined with different instruments.

(12) A. C. Cope and E. Ciganek, *Org. Syn.*, **39**, 40 (1959).

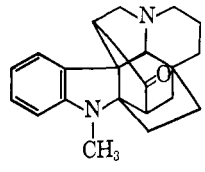
simultaneous oxidation of N_a. The relationship among A, B, and C is however not only established by our own experiments but also by the Swiss group, thus rendering syntheses of structures IX and X unnecessary.

It remains to explain the two well-authenticated facts which, in addition to various pieces of negative evidence, led the Swiss group to the proposal of structures XI, XII, and XIII.

The isolation of pleiocarpinine containing 0.45 atom % of deuterium at C-3, upon treatment of pleiocarpolinine with CH_3OD (99% D) for 5 hr at 170° (as contrasted with 0.26 atom % introduced into pleiocarpinine itself under the same conditions), contradicts in fact the explanation advanced for the facile conversion of pleiocarpolinine into pleiocarpinine. If it were indeed a thermal retroaldol cleavage, as suggested, one should expect the product to have a deuterium content near 99%. The small (but reproducible) excess of deuterium as contrasted to the control (pleiocarpinine) experiment might well be due to a slightly enhanced rate of the exchange in the case of pleiocarpolinine, caused by its greater polarity or a difference in the pH of the solution.

A further result inconsistent with the 3-hydroxymethyl structures was the formation of pleiocarpinine-d_2 when alkaloid B was treated with phosphoric acid-d_3. This product contained no deuterium at C-3 (two deuterium atoms are located in the aromatic nucleus), and was also obtained on treatment of pleiocarpinine itself with the same reagent.[1d]

The reported formation of formaldehyde (0.64 mole isolated as *p*-nitrophenylhydrazone; 0.2 mole determined mass spectrometrically) upon vacuum pyrolysis of pleiocarpoline was confirmed qualitatively also in our laboratory. While it was first thought that it is due to oxidation of methanol of crystallization by the labile oxygen of the N_b-oxide group, the formation of CH_2O upon pyrolysis of a sample of alkaloid A which had been recrystallized from CD_3OH proved that the carbomethoxyl groups are, at least in part, responsible for the formaldehyde. This could involve either prior oxidative (bimolecular) attack on the carbomethoxy group or oxidation of pyrolytically formed methanol. The latter would not be too surprising as we had previously found that the carbomethoxy group at C-3 in pleiocarpinine (and related compounds) can thermally cyclize to C-11, and thus produce 1 mole of methanol in addition to a cyclic ketone. The latter was identical with XIV, a compound synthesized in the course of our work on the structure of 11,22-dioxokopsane,[1c] another *Pleiocarpa* alkaloid.



XIV

Only three naturally occurring amine oxides of indole alkaloids have previously been reported.[7,13,14] Thus, pleiocarpine N_b-oxide (VIII), pleiocarpinine N_b-oxide (IX), and kopsinine N_b-oxide (X) are a major addition to this rare class of compounds.

Experimental Section

Melting points are uncorrected and were taken on a Kofler micro hot stage. Ultraviolet spectra were determined with a Cary Model 14 recording spectrophotometer, and infrared spectra were obtained using a Perkin-Elmer Model 337 spectrophotometer. Nmr spectra were obtained in deuteriochloroform with tetramethylsilane as an internal standard, using a Varian A-60 spectrometer. Mass spectra were determined with a CEC 21-103C mass spectrometer, equipped with a direct inlet system.

Isolation of Alkaloids. The isolation procedure has been described in detail previously.[1b] Combined fractions D34–D40 were rechromatographed on 300 g of silicic acid (100 mesh). Elution with chloroform–methanol (9:1) gave 29 fractions of 100 ml each (chromatogram F).

Alkaloid A (VIII). Combined fractions F21–F23 were crystallized from methanol–ether. Recrystallization from methanol–ether gave 90 mg of white prisms: mp 248–250° dec; $[\alpha]^{26}D$ −131 ± 3° (*c* 2.233, $CHCl_3$); λ_{max}^{MeOH} 244 mμ (log ε 4.08), 281 (3.29), 287 (3.29); $\nu_{max}^{CHCl_3}$ 1730, 1700 cm^{-1}; nmr, Figure 1a, and 3.75 ($NCOOCH_3$), 3.63 ($CCOOCH_3$), 3.33 ppm (CH_3OH). Pleiocarpoline[10] had mp 234–235° dec; $[\alpha]^{24}D$ −131.6 ± 2° (*c* 0.94, $CHCl_3$); λ_{max}^{EtOH} 208 mμ (log ε 4.41), 245 (4.13), 281 (3.33), 287 (3.32); ν_{max}^{KBr} 1739, 1706 cm^{-1}; nmr 8.57 (doublet, J = 7 cps), 7.63 (doublet, J = 7 cps), 7.42–6.8 (multiplet), 3.85 ($NCOOCH_3$), 3.74 ppm ($CCOOCH_3$).

Alkaloid B (IX). Combined fractions F18–F20 were crystallized from methanol–ether. Recrystallization from methanol–ether gave 339 mg of white prisms: mp 239–260° dec; $[\alpha]^{26}D$ −111 ± 2° (*c* 2.575, $CHCl_3$); λ_{max}^{MeOH} 252 mμ (log ε 3.94), 298 (3.45); $\nu_{max}^{CHCl_3}$ 1725 cm^{-1}; nmr, Figure 1b, and 3.48 ($CCOOCH_3$), 3.33 (CH_3OH), 2.68 ppm (NCH_3). Pleiocarpolinine[10] had mp 210–211° dec; $[\alpha]^{22}D$ −111 ± 2° (*c* 0.52, $CHCl_3$); λ_{max}^{EtOH} 207 mμ (log ε 4.42), 251 (4.00), 300 (3.53); λ_{max}^{KBr} 1739 cm^{-1}; nmr 8.29 (doublet, J = 7 cps), 7.45–6.3 (multiplet), 3.80 ($CCOOCH_3$), 2.94 ppm (NCH_3).

Alkaloid C (X). Combined fractions A14–A15[1b] were rechromatographed on thin-layer silica gel H, prewashed with methanol. The major compound (60 mg of alkaloid C) remained amorphous after attempts to crystallize from methanol–ether and acetone–pentane: λ_{max}^{MeOH} 242, 292 mμ; $\nu_{max}^{CHCl_3}$ 1725, 3390 cm^{-1}; nmr, Figure 1c, and 3.70 ($CCOOCH_3$), 3.33 ppm (CH_3OH). Kopsinoline[10] had mp 158–160° dec; $[\alpha]^{22}D$ −70.1°; λ_{max}^{EtOH} 205 mμ (log ε 4.43), 244 (3.87), 295 (3.47); λ_{max}^{KBr} 1727, 3344 cm^{-1}; nmr 8.28 (doublet, J = 7 cps), 7.25–6.3 (multiplet), 3.81 ppm ($CCOOCH_3$).

Catalytic Reduction of Alkaloids A, B, and C. Alkaloid A (38 mg in 4 ml of methanol) was hydrogenated over platinum for 45 min. After filtration and evaporation, the product was crystallized from methanol to give 25 mg of pleiocarpine (I): mp 138–140°; mmp 139–140° with authentic pleiocarpine; $[\alpha]^{25}D$ −142 ± 3° (*c* 1.384, $CHCl_3$). Hydrogenation of alkaloids B and C over platinum gave pleiocarpinine (II) and kopsinine (III), respectively, which were identified by their mass spectra and R_f on tlc.

Lithium Aluminum Hydride and Deuteride Reductions of Alkaloid B. Alkaloid B (5 mg) was dissolved in 2 ml of dry tetrahydrofuran. After addition of excess lithium aluminum hydride, the mixture was stirred for 16 hr at 35°, then evaporated to dryness. Water was added and the product was extracted with chloroform. Tlc indicated a single product, which was identified by its mass spectrum as N-methylkopsinyl alcohol (VII): mass spectrum *m/e* 324 (M^+), 296, 293, 265, 124, 109. The same procedure was followed using lithium aluminum deuteride, giving N-methylkopsinyl alcohol-d_2, mass spectrum *m/e* 326 (M^+), 298, 293, 265, 124, 109.

Ferrous Sulfate Reduction of Alkaloids A and B. In separate experiments, *ca.* 5 mg each of alkaloids A and B was warmed for 15 min at 80° in 1 ml of aqueous ferrous sulfate (10%). The solution was extracted with chloroform, followed by chromatography on thin-layer silica gel. Comparison of R_f values with authentic alkaloids indicated nearly complete conversion of A and B to pleiocarpine (I) and pleiocarpinine (II), respectively.

Pyrolysis of Alkaloid A. Alkaloid A (*ca.* 1 mg) was pyrolyzed directly into the mass spectrometer with a trap at room temperature to permit only the volatile gases to enter the inlet system. A mass spectrum at 9 ev ionizing potential showed molecular ions of methanol (*m/e* 32) and formaldehyde (*m/e* 30). When the sample had been recrystallized from CD_3OH, peaks were observed at *m/e* 30 (CH_2O), 32 (CH_3OH and/or CD_2O), 33 (CD_2OH), and 35 (CD_3OH).

(13) M. Polonovski and C. Nitzberg, *Bull. Soc. Chim. France*, **17**, 244 (1915).

(14) P. R. Ulshafer, W. I. Taylor, and R. H. Nugent, *Compt. Rend.*, **244**, 2989 (1957).

Pyrolysis of Alkaloid B. A vacuum distillation of 25 mg of alkaloid B was performed at 200° (20 μ). After 2 hr, the distillate (5.0 mg) was removed and identified as pleiocarpinine by tlc and its mass spectrum. The residue was separated by tlc into five fractions: (i) 10.5 mg, (ii) 2.1 mg, (iii) 2.1 mg, (iv) 1.3 mg, and (v) 0.5 mg. The mass spectrum of iv was that of pure pleiocarpinine. Fraction ii was identical with N_a-methyl-22-oxokopsane (XIV).[1c] Fraction iii contained 3-isopleiocarpinine, distinguished from pleiocarpinine by the marked intensity differences of peaks *m/e* 124 and 324 in the mass spectrum. These differences are reported to be characteristic of the C-3 epimers.[4] Also present in fraction iii was a dehydrogenated pleiocarpinine (V): mass spectrum *m/e* 350 (M^+), 291, 263, 122, 107. Fractions i and v did not give usable mass spectra, due to thermal decomposition; these compounds may be rearrangement products of the amine oxide IX.

Reaction of Alkaloid B with Phosphoric Acid-d_3. Alkaloid B (5 mg) was refluxed for 1 hr in 1 ml of 50% phosphoric acid-d_3 (from D_2O and P_2O_5). The solution was neutralized with sodium carbonate and extracted with chloroform. The product contained considerable amounts of starting material, together with a small amount of pleiocarpinine-d_2. The latter was isolated by tlc. Both deuterium atoms were in the aromatic nucleus;[1d] mass spectrum *m/e* 354 (M^+), 326, 323, 295, 267, 253, 231, 172, 124, 109.

Oxidation of Pleiocarpine (I) to Pleiocarpine N_b-Oxide (VIII). Pleiocarpine (I, 100 mg) in 0.5 ml of ethanol and 0.5 ml of hydrogen peroxide (31%) was stirred at room temperature for 24 hr. Water (10 ml) was added and the solution was extracted with three 10-ml portions of chloroform. After drying and evaporation of solvent, the product was crystallized from ethanol–ether to give 84 mg of pleiocarpine N_b-oxide (VIII), mp 245–250° dec; mmp 245° with alkaloid A; $[\alpha]^{26}D$ −137 ± 3° (*c* 1.985, $CHCl_3$); λ_{max}^{MeOH} 206 mμ (log ε 4.46), 244 (4.13), 280 (3.30), 287 (3.27); infrared and nmr spectra identical with spectra of alkaloid A, except for the lack of a peak at 3.33 ppm (CH_3OH). No analogous signal could be detected in the nmr spectrum for ethanol; the compound appears to be a hydrate. Upon recrystallization from methanol–ether, the product exhibited an nmr spectrum identical with that of alkaloid A.

Acknowledgment. We thank Dr. F. A. Hochstein (Chas. Pfizer & Co.) for the crude bark extract. This investigation was supported by a Public Health Service Fellowship (5-F1-GM-21,624-03) from the National Institute of General Medical Sciences (to D. W. T.) and a research grant from the National Science Foundation (GP-3734).

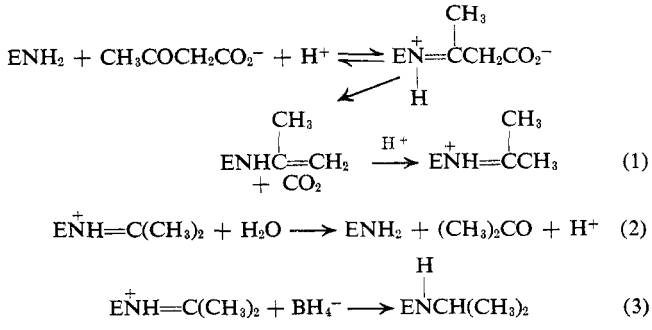
The Active Site of Acetoacetate Decarboxylase[1]

Richard A. Laursen[2] and F. H. Westheimer

Contribution from the James Bryant Conant Laboratory of Harvard University, Cambridge, Massachusetts. *Received February 3, 1966*

Abstract: Sodium borohydride reduces a mixture of acetoacetic acid, labeled in the 3 position with ^{14}C, and acetoacetate decarboxylase to incorporate radiocarbon into the enzyme. Hydrolysis of the resulting protein with trypsin has led to the formation of a single radioactive peptide, which contains ε-N-isopropyllysine. (This amino acid arises from reduction of a Schiff base between the enzyme and decarboxylated substrate.) Similarly, a single radioactive peptide could be isolated after digestion with chymotrypsin. Sequence analysis of these peptides shows that the active site of the enzyme has the structure -Glu-Leu-Ser-Ala-Tyr-Pro-Lys*-Lys-Leu-, where Schiff base formation and borohydride reduction occur at the starred lysine residue.

The decarboxylation of acetoacetic acid is catalyzed by a crystalline decarboxylase from *Clostridium acetobutylicum*.[3,4] The reaction proceeds by the formation of a Schiff base between the enzyme and acetoacetate, followed by decarboxylation to form an enamine; the enamine is in turn protonated to form the Schiff base salt of acetone, and then hydrolysis regenerates enzyme and liberates product.[5]



This mechanism (eq 1 and 2) was first postulated in 1959 on the basis of the discovery[6] that the enzymic decarboxylation involves the obligatory exchange of the carbonyl oxygen atom of acetoacetate with oxygen atoms of the solvent (water). It was confirmed by the observation[7] that the reduction of a solution containing enzyme and acetoacetate with sodium borohydride[8] leads to inactivation of the enzyme. When the reaction was carried out with acetoacetate labeled in the 3 position with ^{14}C, the resulting protein was radioactive, and on hydrolysis yielded a single radioactive amino acid. This amino acid has beeen identified as ε-N-isopropyllysine,[5,9] and it has been shown that it arises

(1) This work was supported by the National Institutes of Health under Grant GM-04712.

(2) National Institutes of Health Postdoctoral Fellow, 1964–1966, 5-F2-GM-17,608-02.

(3) G. Hamilton and F. H. Westheimer, *J. Am. Chem. Soc.*, **81**, 2277 (1959).

(4) B. Zerner, S. M. Coutts, F. Lederer, H. H. Waters, and F. H. Westheimer, *Biochemistry*, **5**, 813 (1966).

(5) F. H. Westheimer, *Proc. Chem. Soc.*, 253 (1963).

(6) G. Hamilton and F. H. Westheimer, *J. Am. Chem. Soc.*, **81**, 6332 (1959).

(7) I. Fridovich and F. H. Westheimer, *ibid.*, **84**, 3208 (1962).

(8) See E. H. Fischer in "Structure and Activity of Enzymes," T. W. Goodwin, J. I. Harris, and B. S. Hartley, Ed., Academic Press Inc., New York, N. Y., 1964, p 111, for a review of borohydride reductions of enzymic systems.

(9) S. Warren, B. Zerner, and F. H. Westheimer, *Biochemistry*, **5**, 817 (1966).