bound to the enzyme.[12] The amount of bound Mn^{2+} is reduced by back-titrating with the diamagnetic Mg^{2+} resulting in a narrowing of ^{205}Tl line. The line width was reduced from 30.5 to 25 Hz in the presence of 5×10^{-3} *M* $Mg(NO_3)_2$ and 4.7×10^{-4} *M* $Mn(NO_3)_2$. Apparently, bound Mg^{2+} has no effect on the line width of bound $^{205}Tl^+$. The width was further reduced to 20.5 Hz by back-titrating the bound Tl^+ itself with K^+ at a final concentration of 0.5 *M* in KNO_3. The control experiments show that the observed phenomena result from the binding of Tl^+ to the enzyme and the interaction of bound $^{205}Tl^+$ with the bound Mn^{2+} ions. The fact that no additional broadening is produced by the diamagnetic Mg^{2+} strongly suggests that the factor that changes in the presence of Mn^{2+} is the relaxation rate, $1/T_{2M}$, of bound $^{205}Tl^+$ due to dipolar interactions with the unpaired electronic spin of Mn^{2+}. An additional broadening (upon adding Mn^{2+} to the Tl–PK solution) of the observed magnitude could only occur if the system were originally in the limit of fast exchange [case b], but now, owing to the increase in $1/T_{2M}$, the condition $1/T_{2M} \gg \Delta\omega_M$ prevails. Thus, the upper limit for τ_M is set now at 1.7×10^{-5} sec, which is also the upper limit for T_{2M}. The correlation time for the dipolar interaction is determined predominantly by the shortest among the rotational correlation time, the electron spin relaxation time, and the mean lifetime of the complex. Recent proton relaxation studies[12] indicate that the magnitude of the relevant correlation time is approximately 10^{-8} sec. We have used the well-known relation[13]

$$1/T_{2M} = (1/15)S(S + 1)\gamma_I{}^2g^2\beta^2r^{-6}\, f(\tau_c),$$

where $f(\tau_c) = \tau_c[4 + 3/(1 + \omega_I{}^2\tau_c{}^2) + 13/(1 + \omega_s{}^2\tau_c{}^2)]$, with $T_{2M} = 1.7 \times 10^{-5}$ sec and correlation times of 10^{-9}, 10^{-8}, and 5×10^{-8} sec and obtained values of 4.23, 5.85, and 7.40 Å, respectively, for the distance (r) between the interacting nuclear (^{205}Tl) and electronic (of Mn^{2+}) spins. (The calculated distance is relatively insensitive to the various assumptions since it is dependent on the sixth root of $f(\tau_c)T_{2M}$.) These tentative figures representing the upper limit indicate that the monovalent and divalent binding sites are in very close proximity and thus the monovalent as well as the divalent[4] metal ion activators could directly participate in the catalysis of the pyruvate kinase reaction as suggested recently by Suelter.[14] For example, the distance between the phosphate and carboxylate coordination sites of phosphoenol pyruvate has recently been estimated at approximately 6 Å.[15] These observations seem to contradict the generally held concept[5] that monovalent cation activation is due mainly to conformational changes stabilized by the otherwise passive monovalent ions. Although this role is not ruled out by the present study, the site of action would then have to be very near the active site itself.

The effects of temperature and added substrates on the ^{205}Tl line broadening due to Mn^{2+} are currently under investigation and the results will be published in due course.

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(12) J. Reuben and M. Cohn, to be published. *Cf.* also G. L. Cottam and R. L. Ward, *Arch. Biochem. Biophys.*, **132**, 308 (1969).

(13) I. Solomon, *Phys. Rev.*, **99**, 559 (1955); A. Abragam, "The Principles of Nuclear Magnetism," Oxford University Press, London, 1961, Chapter 8; see also R. E. Connick and D. Fiat, *J. Chem. Phys.*, **44**, 4103 (1966).

(14) C. H. Suelter, Abstracts, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, BIOL. 056.

(15) A. S. Mildvan, J. R. Hunsley, and C. H. Suelter in "Johnson Foundation Symposium on Probes for Macromolecular Structure and Function," B. Chance, R. Yonetani, and M. Cohn, Ed., in press.

(16) On leave from the Weizmann Institute of Science, Rehovoth, Israel. This work was done during the tenure of a Career Investigator Fellowship of the American Heart Association.

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Alkaloid Studies. LXII.[1] X-Ray Crystallographic Structure Determination of Dichotine Hydrobromide

Sir:

In 1965 the isolation from *Vallesia dichotoma* Ruiz *et* Pav of 28 alkaloids containing a remarkable variety of indole structural types was reported.[2] All but six of them were identified by physical and analytical methods and by chemical correlation with known compounds. Of the remaining alkaloids, four were available in sufficient amount for further investigation. Two of them, (+)-vallesiachotamine[3] and (−)-vallesamidine,[4] were subsequently shown by us to have biogenetically very intriguing structures and thus provided additional stimulus to elucidate the constitution of the last two alkaloids (alkaloids **26** and **25** in ref 2, now named dichotine and 11-methoxydichotine), which exhibit an unusually high degree of oxygenation.

(+)-Dichotine ($C_{22}H_{26}N_2O_6$, I, R = H) and (+)-11-methoxydichotine ($C_{23}H_{28}N_2O_7$, I, R = OCH_3) differ from each other by a methoxyl group on the benzene ring. Using extensive chemical and spectroscopic data, the two alkaloids were found[5] to encompass partial formulation II, which contains several structural features hitherto unencountered in the field of indole alkaloids. Since the supply of these rare alkaloids was virtually exhausted, the complete structure was determined by X-ray diffraction analysis of dichotine hydrobromide.

Dichotine hydrobromide was crystallized from absolute ethanol to give orthorhombic hexahedrons. The space group is $P2_12_12_1$ with unit cell dimensions of a = 14.017 ± 0.005, b = 17.241 ± 0.005, c = 9.913 ± 0.005 Å; V = 2395.6 Å^3. The density (measured by flotation) and microanalysis indicate four molecules of alkaloid and four molecules of ethanol per unit cell (calculated density: 1.500 ± 0.001 g/cm^3; found: 1.498 ± 0.005 g/cm^3. *Anal.* Calcd for $C_{24}H_{33}N_2O_7Br$: C, 53.23; H, 6.15; N, 5.17; Br, 14.76. Found: C, 52.95; H, 6.24; N, 5.44; Br, 15.07.)

A total of 21,816 diffraction intensities was collected by a Hilger–Watts computer-controlled Y290 diffractometer, using monochromatic Cu Kα radiation. Averaging according to Friedel's law gave 2773 unique

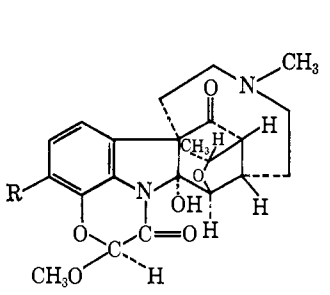
(1) For paper LXI see B. V. Milborrow and C. Djerassi, *J. Chem. Soc., C*, 417 (1969).

(2) A. Walser and C. Djerassi, *Helv. Chim. Acta*, **48**, 391 (1965).

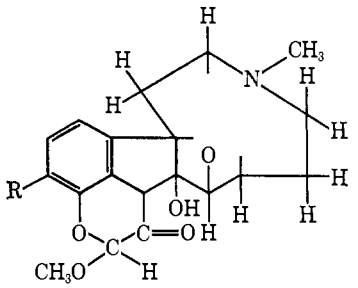
(3) H. J. Monteiro, A. Walser, L. J. Durham, and C. Djerassi, *J. Am. Chem. Soc.*, **88**, 1792 (1966).

(4) S. H. Brown, C. Djerassi, and P. G. Simpson, *ibid.*, **90**, 2445 (1968).

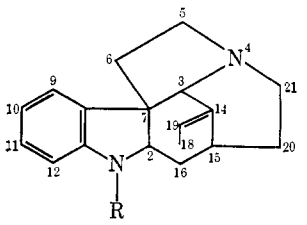
(5) N. C. Ling and C. Djerassi, paper in preparation.



I

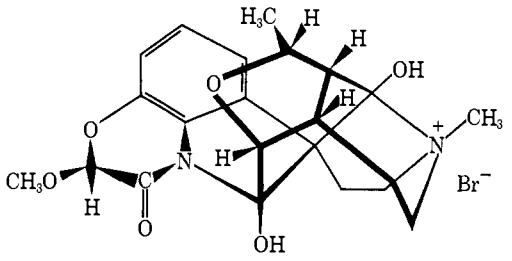


II



III

reflections, of which 2757 were not systematically extinct. The unique bromine position was determined from a sharpened three-dimensional Patterson function. The reliable image method,[6] a new systematic and objective analysis of the Patterson function, was used to derive a trial image of the remaining 33 light atoms. However, 2 of the 33 atoms were eliminated by the least-squares refinement, but their new positions were located in the difference Fourier map. After several full-matrix least-squares refinements with all the data, the discrepancy factor ($R = \Sigma(||F_o| - |F_c||)/\Sigma|F_o|$) was lowered to 12.6%.



A

A three-dimensional projection of dichotine hydrobromide, showing its relative configuration, is presented (A). In the free base the N_b nitrogen is bonded to the carbonyl carbon to give a zwitterion. This zwitterionic character has precedence in the Strychnos alkaloids such as (+)-vomicine,[7] (−)-novacine,[8] and (−)-icajine.[9] As a result, the hydrobromide of dichotine is protonated on the carbonyl oxygen instead of the N_b nitrogen and a full-fledged carbon–nitrogen bond is formed between the N_b nitrogen and the carbonyl carbon.

Biogenetically, the overall skeleton of dichotine (I, R = H) and 11-methoxydichotine (I, R = OCH_3) can be derived from aspidospermatidine[10] (III, R = H) by oxidative cleavage of the 3,4 bond,[11] followed by N-methylation. This biosynthetic route is supported by the isolation of N-acetylaspidospermatidine[2] (III, R = $COCH_3$) from the same plant. While relatively simple steps can be envisaged for the biogenetic introduction of the tetrahydrofuran ring, the most unusual feature in these two alkaloids is the six-membered acetal amide ring which has not been encountered as yet in any indole alkaloids.

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(6) J. W. Becker and P. G. Simpson, paper in preparation.
(7) H. Wieland and G. Oertel, *Ann. Chem.*, **469**, 193 (1929); R. Huisgen, H. Eder, L. Blazejewicz, and E. Mergenthaler, *ibid.*, **573**, 121 (1951).
(8) W. F. Martin, H. R. Bentley, J. A. Henry, and F. S. Spring, *J. Chem. Soc.*, 3603 (1952).
(9) H. Bisset, *C. R. Acad. Sci., Paris*, **261**, 5237 (1965).
(10) K. Biemann, A. L. Burlingame, and D. Stauffacher, *Tetrahedron Lett.*, 527 (1962); K. Biemann, M. Friedmann-Spiteller, and G. Spiteller, *ibid.*, 485 (1961).
(11) For numbering system see M. Hesse, "Indoalkaloide in Tabellen," Vol. I, Springer-Verlag, Berlin–Gottingen–Heidelberg, 1964, p 41.

A Higher Oxidation State for a Coupled Icosahedral Borane Anion[1]

Sir:

The only known compounds having two B_{12} or $B_{10}C_2$ icosahedra linked directly are biscarborane ($B_{20}C_4H_{22}$)[2] and some partially halogenated derivatives of the as yet unobserved $B_{24}H_{22}^{4-}$ ion, which would be isoelectronic with biscarborane. In addition, the known $B_{24}H_{23}^{3-}$ ion (and its partially halogenated derivatives) can be regarded as protonated $B_{24}H_{22}^{4-}$.[3] All of these compounds can be represented as resulting from a one-electron oxidation of the icosahedral $B_{12}H_{12}^{2-}$ or $B_{10}C_2H_{12}$, followed by dimerization and elimination of H^+. Although the $B_{24}H_{23}^{3-}$ ion is itself resistant to further oxidation without degradation, some of its partially halogenated derivatives are more easily oxidized.[3] We wish to report the oxidation of $B_{24}H_{21}I_2^{3-}$ to $B_{24}H_{20}I_2^{2-}$, a derivative of the higher oxidation state ion, $B_{24}H_{22}^{2-}$, which has not yet been observed.

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(2) J. A. Dupont and M. F. Hawthorne, *J. Amer. Chem. Soc.*, **86**, 1643 (1964).
(3) R. J. Wiersema and R. L. Middaugh, *Inorg. Chem.*, **8**, 2074 (1969).