University of Massachusetts Computing Center for generous allocation of computer time.

Supplementary Material Available: Tables of calculated coordinates for UpcA and UpA (ESC-A, ESC-B, CI-A, CI-B, TS-A, TS-B, and the cyclic intermediate with the best lysine-41 position) (8 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) Pentacoordinated Molecules. 28. Paper presented in part at the 174th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1977, Abstract No. BIOL 9; (b) for part 27, see R. R. Holmes, *J. Am. Chem. Soc.*, **100**, 433 (1978).
(2) This work represents in part a portion of the Ph.D. thesis of J. C. Gallucci, University of Massachusetts, Amherst, Mass.
(3) (a) A. S. Mildvan, *Acc. Chem. Res.*, **10**, 246 (1977); (b) A. S. Mildvan and M. Cohn, *Adv. Enzymol.*, **33**, 1 (1970).
(4) (a) R. M. Sweet, H. T. Wright, J. Janin, C. H. Chothia, and D. M. Blow, *Biochemistry*, **13**, 4212 (1974); (b) R. Huber, D. Kukla, W. Bode, P. Schwager, K. Bartels, J. Deisenhofer, and W. Steigemann, *J. Mol. Biol.*, **89**, 73 (1974); (c) R. Huber, W. Bode, D. Kukla, U. Kohl, and C. A. Ryan, *Biophys. Struct. Mech.*, **1**, 189 (1975); (d) F. A. Cotton and E. E. Hazen, Jr., "The Enzymes", 3rd ed., Vol. IV, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1971, pp 153–174.
(5) (a) K. E. B. Platzer, F. A. Momany, and H. A. Scheraga, *Int. J. Pept. Protein Res.*, **4**, 187, 201 (1972); (b) G. M. Lipkind and M. Ya. Karpeiskii, *Mol. Biol. (Moscow)*, **10**, 395 (1976); (c) E. M. Popov, *ibid.*, **11**, 5 (1977); (d) I. B. Golovanov, V. M. Sobolev, E. V. Shchukina, and M. V. Vol'kenshtein, *ibid.*, **10**, 7703 (1976).
(6) F. M. Richards and H. W. Wyckoff in ref 4d, Chapter 24, pp 647–806.
(7) See also R. R. Holmes, *Int. J. Pept. Protein Res.*, **8**, 445 (1976), and references cited therein.
(8) G. C. K. Roberts, E. A. Dennis, D. H. Meadows, J. S. Cohen, and O. Jardetzky, *Proc. Natl. Acad. Sci. U.S.A.*, **62**, 1151 (1969).
(9) (a) N. L. Allinger, M. T. Tribble, M. A. Miller, D. H. Wertz, *J. Am. Chem. Soc.*, **93**, 1637 (1971); (b) D. H. Wertz and N. L. Allinger, *Tetrahedron*, **30**, 1579 (1974).
(10) J. A. Deiters, J. C. Gallucci, T. E. Clark, and R. R. Holmes, *J. Am. Chem. Soc.*, **99**, 5461 (1977).
(11) (a) R. R. Holmes and J. A. Deiters, *J. Am. Chem. Soc.*, **99**, 3318 (1977); (b) *J. Chem. Res.* (S), 92 (1977).
(12) J. E. Williams, P. J. Stang, and P. v. R. Schleyer, *Annu. Rev. Phys. Chem.*, **19**, 531 (1968), have reviewed the calculational methods of conformation analyses.
(13) F. M. Richards and H. W. Wyckoff, "Atlas of Molecular Structures in Biology", Vol. 1, D. C. Phillips and F. M. Richards, Eds., Clarendon Press, Oxford, 1973.
(14) B. Walter and F. Wold, *Biochemistry*, **15**, 304 (1976).
(15) J. L. Sussman and N. C. Seeman, *J. Mol. Biol.*, **66**, 403 (1972).
(16) R. M. Badger, *J. Chem. Phys.*, **2**, 128 (1934).
(17) S. Arnott, *Prog. Biophys. Mol. Biol.*, **21**, 274–275 (1970).
(18) Op. cit. in ref 7.
(19) C. L. Coulter, *J. Am. Chem. Soc.*, **95**, 570 (1973).
(20) G. M. Lipkind and M. Ya. Karpeiskii, *Dokl. Akad. Nauk SSSR*, **224** (No. 5), 1212 (1975).
(21) Op. cit. in ref 6, p 788.
(22) D. A. Usher, D. I. Richardson, Jr., and F. Eckstein, *Nature (London)*, **228**, 663 (1970).
(23) D. A. Usher, E. S. Erenrich, and F. Eckstein, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 115 (1972).
(24) D. B. Boyd, *J. Am. Chem. Soc.*, **91**, 1200 (1969).

Communications to the Editor

^2H NMR as a Probe of the Stereochemistry of Enzyme Reactions at Prochiral Centers. Deamination of Cadaverine, Catalyzed by Diamine Oxidase

Sir:

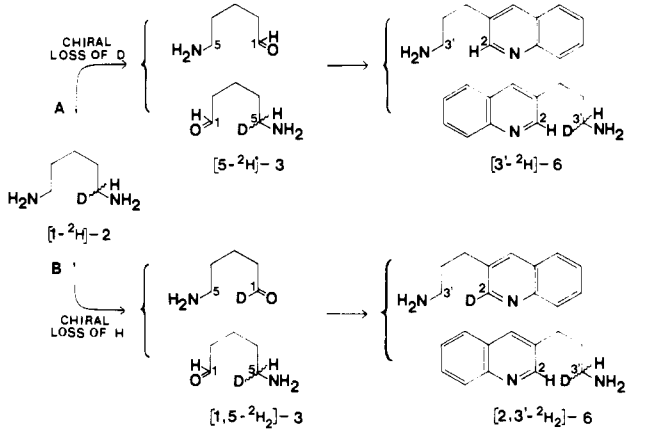
Diamine oxidase (E.C. 1.4.3.6, diamine:oxygen oxidoreductase (deaminating)) catalyzes the oxidative deamination of a wide range of primary amines, including diamines, histamine, and arylalkylamines.[1-3] The aliphatic diamines, cadaverine and putrescine, are the substrates which are most readily oxidized. Thus, cadaverine (**2**), by loss of one of the two equivalent amino groups, yields 5-aminopentanal (**3**), which is in equilibrium with its cyclized form, Δ^1-piperideine (**4**).[4-6] It is to be expected that the reaction takes place in a sterically controlled manner. The oxidation of histamine, catalyzed by hog kidney diamine oxidase,[7] and that of benzylamine, mediated by diamine oxidase from pea seedlings,[8] takes place with stereospecific release of one of the two enantiotopic hydrogen atoms from the carbon adjacent to nitrogen. In the latter case, the stereochemistry of the reaction was determined by means of tritium labeling and determination of $^3H/^{14}C$ ratios. The chirality of the analogous oxidation of tyramine, catalyzed by monoamine oxidase (E.C. 1.4.3.4) of rat liver, was determined by a kinetic method, using deuterium labeled substrates.[9]

The stereochemistry of the oxidative deamination of cadaverine has not been established. We have employed ^2H NMR spectrometry to demonstrate that, in the course of this reaction, catalyzed by diamine oxidase of hog kidney (E.C. 1.4.3.6), it is the *pro-S* hydrogen which is removed stereospecifically from the carbon atom adjacent to the reacting amino group.

The enantiomers of [1-^2H]cadaverine, required for the study, were prepared by decarboxylation of L-lysine (**1**), catalyzed by L-lysine decarboxylase (E.C. 4.1.1.18, L-lysine carboxylyase) from *Bacillus cadaveris*, a reaction which is known[10] to proceed with retention of configuration. (+)-(*S*)-[1-^2H]Cadaverine dihydrochloride (**2A**) (>95% deuterated at the *pro-S* position of one of the terminal carbon atoms)[11] was obtained from the L-component of DL-[2-^2H]-lysine.[12] (−)-(*R*)-[1-^2H]Cadaverine dihydrochloride (**2B**) (>95% deuterated at the *pro-R* position of one of the terminal carbon atoms)[11] was obtained by decarboxylation of L-lysine in deuterium oxide (99.7 atom %, Merck Sharp and Dohme). A third deuterated cadaverine, [1,1-$^2H_2$]cadaverine (**2C**) (>95% perdeuterated at one of the terminal C atoms),[11] was prepared by decarboxylation of DL-[2-^2H]lysine in deuterium oxide.

In separate experiments, each of the three deuterated samples of cadaverine (40 mg) was incubated (37 °C, 40 h) with hog kidney diamine oxidase (Sigma Grade II, 200 mg),

Scheme I



0002-7863/78/1500-7402$01.00/0

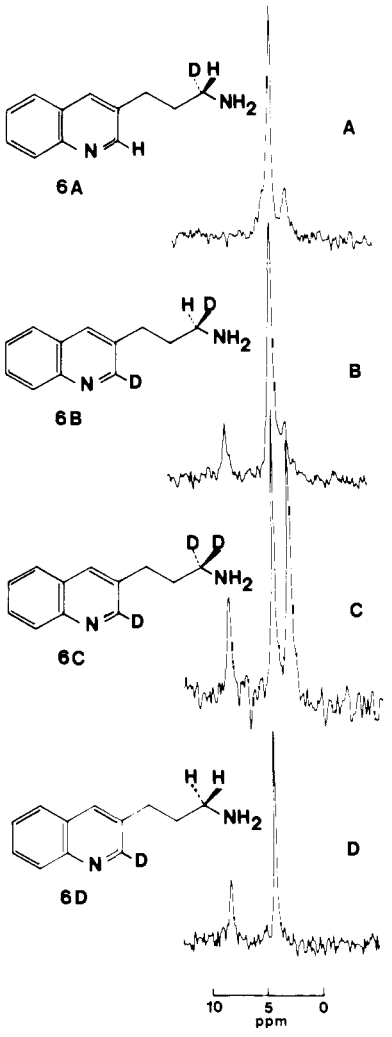


Figure 1. Proton decoupled ^2H NMR spectra of deuterium labeled samples of 3-(3′-aminopropyl)quinoline dihydrochloride (**6**): A (**6A**), derived from (*S*)-[1-^2H]cadaverine (**2A**) (via enzymic oxidative deamination) (5 mM, 56 628 transients); B (**6B**), derived from (*R*)-[1-^2H]cadaverine (**2B**) (5 mM, 57 000 transients); C (**6C**), derived from [1,1-$^2H_2$]cadaverine (**2C**) (13 mM, 3475 transients); D (**6D**), obtained from DL-[2-^2H]lysine by chemical oxidation[18] (8 mM, 4808 transients).

together with beef liver catalase[14] (E.C. 1.11.1.6, H_2O_2:H_2O_2 oxidoreductase) (Sigma, 0.5 mg) and *o*-aminobenzaldehyde (**5**, 32 mg). The latter traps the product of the enzymic reaction, 5-aminopentanal (**3**), to yield 3-(3′-aminopropyl)quinoline (**6**).[15]

The location of deuterium in the samples of **6** obtained from the three deuterated cadaverines **2A**, **2B**, and **2C** was determined by ^2H NMR spectroscopy.[16]

Oxidation of a chirally deuterated [1-^2H]cadaverine with stereospecific loss of deuterium leads to a sample of 5-aminopentanal (**3**), enriched in deuterium exclusively at C-5, together with nondeuterated (**3**). The quinoline derivative (**6**) obtained from this 5-[5-^2H]aminopentanal will be deuterated exclusively at C-3′ of the side chain (Scheme I, A). Oxidation of a chirally deuterated [1-^2H]cadaverine, with stereospecific retention of deuterium but loss of protium from C-1, leads to a sample of 5-aminopentanal, intermolecularly doubly labeled at C-1 and C-5. This, in turn, yields an intermolecularly labeled quinoline derivative, deuterated at C-3′ of the side chain and at C-2 of the nucleus (Scheme I, B).

Assignment of NMR signals due to ^1H and ^2H at these two positions was made using compounds of known isotope distribution: The ^1H NMR[17] signals assigned to the protons at C-3′ and C-2 of undeuterated **6** appeared at δ 2.95 (t, J = 7.2 Hz) and 8.68 (s, br) ppm, respectively. The downfield signal (δ 8.68 ppm) was absent in the ^1H NMR spectrum of a sample of 3-[2-^2H](3′-aminopropyl)quinoline (**6D**).[18]

The ^2H NMR[16] spectrum of **6D** showed a single peak at δ 8.8 ppm (Figure 1D) (apart from the signal at δ 4.5 ppm, due to the natural abundance of deuterium in water ($^1H^2HO$) present in all spectra in Figure 1). The ^2H NMR spectrum of [2,3′,3′-$^2H_3$]-**6** (**6C**), derived from [1,1,-$^2H_2$]cadaverine (**2C**) showed two signals at δ 2.9 and 8.8 ppm, of relative intensity 2:1, due to deuterium at C-3′ and C-2 (Figure 1C).

The spectra given by the quinoline derivatives obtained from the two enantiomeric [1-^2H]cadaverines are shown in Figures 1A and 1B. The quinoline derived from (*S*)-[1-^2H]cadaverine (**2A**) showed a single ^2H NMR signal at δ 2.9 ppm (Figure 1A), indicating that only C-3′ was labeled with deuterium. The quinoline from (*R*)-[1-^2H]cadaverine (**2B**), on the other hand, showed two signals at δ 2.9 and 8.8 ppm, in the ratio 1:1 (Figure 1B), due to the presence of deuterium at C-2 as well as at C-3′.

It follows that diamine oxidase (E.C. 1.4.3.6) from hog kidney mediates the stereospecific removal of the *pro-S* hydrogen from C-1 of cadaverine, and that the product of oxidative deamination, 5-aminopentanal (**3**), in equilibrium with Δ^1-piperideine (**4**), retains the *pro-R* hydrogen at the sp^2 carbon.

This stereospecificity corresponds to that of the oxidative deamination of benzylamine to benzaldehyde, catalyzed by the diamine oxidase (E.C. 1.4.3.6) of pea seedlings, which also involves loss of the *pro-S* hydrogen.[8] The analogous oxidation of tyramine, catalyzed by monoamine oxidase (E.C. 1.4.3.4) from rat liver, on the other hand, takes place with loss of the *pro-R*-hydrogen atom from the carbon adjacent to the amino group.[9]

Acknowledgment. This work was supported by the National Research Council of Canada.

References and Notes

(1) E. A. Zeller, *Enzymes*, 2nd ed., **8**, 313 (1963).
(2) J. M. Hill and P. J. G. Mann, *Biochem. J.*, **91**, 171 (1964).
(3) W. G. Bardsley, C. M. Hill, and R. W. Lobley, *Biochem. J.*, **117**, 169 (1970).
(4) H. Tabor, *J. Biol. Chem.*, **188**, 125 (1951).
(5) K. Hasse and H. Maisack, *Biochem. Z.*, **327**, 296 (1955).
(6) P. J. G. Mann and W. R. Smithies, *Biochem. J.*, **61**, 89 (1955).
(7) C. M. Hill and W. G. Bardsley, *Biochem. Pharmacol.*, **24**, 253 (1975).
(8) A. R. Battersby, J. Staunton, and M. C. Summers, *J. Chem. Soc., Chem. Commun.*, 548 (1974); *J. Chem. Soc., Perkin Trans. 1*, 1052 (1976).
(9) B. Belleau, M. Fang, J. Burba, and J. Moran, *J. Am. Chem. Soc.*, **82**, 5752 (1960).
(10) E. Leistner and I. D. Spenser, *J. Chem. Soc., Chem. Commun.*, 378 (1975).
(11) Isotopic abundance of the samples of [1-^2H]- and [1,1-$^2H_2$]cadaverine was determined by ^1H NMR. Optical rotation of the samples of (*R*)-and (*S*)-[1-^2H]cadaverine dihydrochloride was measured using a Perkin-Elmer 241 MC polarimeter (1-dm tubes, 26 °C): (*R*)-[1-^2H]cadaverine dihydrochloride (*c*, 49.2 mg/mL in 0.1 N HCl), $[\alpha]_{546}$ −0.14 ± 0.02°, $[\alpha]_{405}$ −0.23 ± 0.03°; (*S*)-[1-^2H]cadaverine dihydrochloride (*c*, 31.7 mg/mL in 0.1 N HCl), $[\alpha]_{546}$ +0.15 ± 0.03°, $[\alpha]_{405}$ +0.25 ± 0.03° (uncorrected for unlabeled cadaverine dihydrochloride).
(12) DL-[2-^2H]Lysine (>98% deuterated at C-2, as determined by ^1H NMR) was prepared (cf. ref 13) by reaction of *N*-(4-bromobutyl)phthalimide with sodium diethyl acetamidomalonate in ethanol, followed by hydrolysis of the substitution product, diethyl 2-acetamido-2-(4-phthalimidobutyl)malonate, in deuterium chloride (38% w/w in D_2O) and isolated as its monohydrochloride.
(13) M. Fields, D. E. Walz, and S. Rothchild, *J. Am. Chem. Soc.*, **73**, 1000 (1951).
(14) Catalase mediates the disproportionation to oxygen and water of the hydrogen peroxide formed during the enzymic oxidation of the amine.
(15) C. Schöpf, A. Komzak, F. Braun, and E. Jacobi, *Justus Liebigs Ann. Chem.*, **559**, 1 (1948).
(16) ^2H NMR spectra were recorded on a Bruker WH90 pulsed Fourier transform spectrometer operating at 13.82 MHz at ambient temperature, with broad band proton noise decoupling. All spectra were obtained from aqueous solutions (1 mL) in 10-mm-o.d. sample tubes using ^2H_2O as external lock: repetition time, 1.024 s; pulse time, 15 μs. The natural abundance deuterium signal of $^1H^2HO$ served as internal standard. Chemical shifts are referenced to perdeuteriotetramethylsilane.

(17) ^{1}H NMR spectra at 90 MHz were of solutions of **6**, and **6D** dihydrochlorides in 0.2 M perdeuterioacetate buffer in deuterium oxide (pD ~5.2).
(18) 3-[2-^{2}H](3'-Aminopropyl)quinoline (**6D**) dihydrochloride was obtained in two steps from DL-[2-^{2}H]lysine. Oxidation with *N*-bromosuccinimide gave Δ^1-[2-^{2}H]piperideine,[19] in equilibrium with 5-[1-^{2}H]aminopentanal, which was condensed with *o*-aminobenzaldehyde.[15]
(19) R. N. Gupta and I. D. Spenser, *Can. J. Chem.*, **47**, 445 (1969).

James C. Richards, Ian D. Spenser*
Department of Chemistry, McMaster University
Hamilton, Ontario, Canada L8S 4M1
Received July 25, 1978

Energy Transfer Processes Involving Distorted Excited States

Sir:

Some years ago, Weller[1,2] extended the Marcus theory[3-6] to electron-transfer quenching processes. Recent results concerning excited-state electron-transfer processes of organic[7,8] and inorganic[9-11] molecules have confirmed the validity of that treatment.[12] We will show here that the same concepts can be used to treat collisional energy-transfer processes,[13] and that in this way it is possible to obtain a unified view of "classical" and "nonclassical" energy-transfer behavior.[14-21]

For an energy-transfer quenching process involving a collisional (exchange) mechanism,[14] this kinetic scheme 1 can be

$$\begin{array}{ccccccc} {}^*D + Q & \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} & {}^*D\cdots Q & \underset{k_{-en}}{\overset{k_{en}}{\rightleftharpoons}} & D\cdots {}^*Q & \underset{k_d}{\overset{k_{-d}}{\rightleftharpoons}} & D + {}^*Q \\ \downarrow 1/\tau_{*D} & & & & & & \downarrow 1/\tau_{*Q} \end{array} \quad (1)$$

used. Assuming that $1/\tau_{*Q} \gg k_d[D]$ and using Stern-Volmer relationships and steady-state approximations, the experimental quenching constant k_q is given by

$$k_q = \frac{k_d}{1 + \frac{k_{-d}}{k_{en}}\left(1 + \frac{k_{-en}}{k_{-d}}\right)} \quad (2)$$

Scheme 1 and eq 2 are analogous to the scheme and the equation obtained for electron-transfer quenching.[1,2,7-12] When at least one of the species involved is uncharged, no work is required to bring the reactants or products together at the separation distance of the encounter complex, and thus the free activation energy and the free-energy change of the whole process coincide with the analogous quantities ($\Delta G^{\ddagger}$ and ΔG) involved in the energy-transfer step. As $k_{-en}/k_{en} = \exp(\Delta G/RT)$ and $k_{en} = k_{en}^0 \exp(-\Delta G^{\ddagger}/RT)$, where k_{en}^0 is the frequency factor of the energy-transfer step, eq 2 can be transformed into

$$k_q = \frac{k_d}{1 + e^{\Delta G/RT} + \frac{k_{-d}}{k_{en}^0} e^{\Delta G^{\ddagger}/RT}} \quad (3)$$

The meaning of free energy in excited-state reactions and the relationships between spectroscopic and thermodynamic quantities have been thoroughly discussed by Grabowski.[22] The free-energy difference of the energy-transfer process can be expressed by

$$\Delta G = -E^{0-0}(*D,D) + E^{0-0}(*Q,Q) + C(*D,D) + C(Q,*Q) \quad (4)$$

where E^{0-0} is the zero-zero excited-state energy and C is a term which accounts for the difference in partition functions and entropy between ground and excited state. C can be either positive or negative and in favorable cases it is very small and can thus be neglected.[2,22]

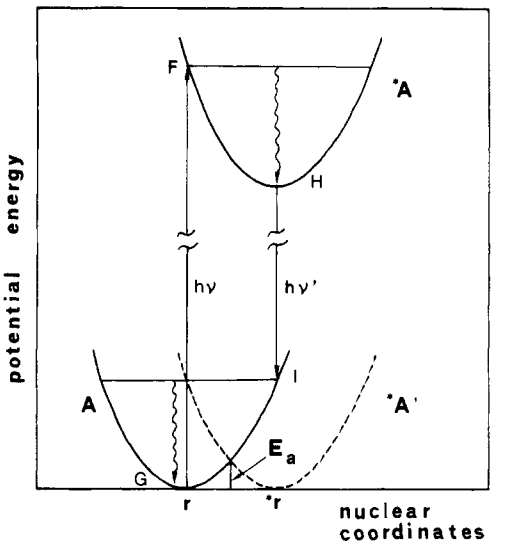


Figure 1. Schematic potential energy diagram for a molecule having the excited state with different nuclear coordinates. For the sake of simplicity, the two states are assumed to be described by the same harmonic function and the zero point energy is neglected. The Stokes shift (S) is given by FG-HI. In this very simple case, the "intrinsic barrier" to energy transfer caused by excited-state distorsion, E_a, is equal to $\frac{1}{8}S$.

In order to understand the meaning of $\Delta G^{\ddagger}$, consider a "self-exchange" energy-transfer reaction

$$*A + A \rightarrow A + *A \quad (5)$$

between an excited state and a ground state having minima at different values of the nuclear coordinates (Figure 1). For the sake of simplicity, the two states are assumed to be described by the same harmonic function. A collision between *A and A when both are in their zero vibrational levels cannot result in energy transfer because of Franck-Condon restrictions and energy conservation. The energy deficiency can be made up by vibrational excitation of *A and/or A. The most convenient reaction path is that involving excitation of both *A and A to vibrational levels which correspond to the crossing point between curves A and *A' in Figure 1. For each molecule we can thus define a quantity (E_a in Figure 1) which is related to excited-state distorsion and represents an "intrinsic barrier" to energy transfer. The free activation energy of a "self-exchange" energy-transfer reaction like that in eq 5 can thus be expressed by

$$\Delta G^{\ddagger}_A = 2(E_a + C^{\ddagger}) \quad (6)$$

where E_a is in some way related to the Stokes shift and $C^{\ddagger}$ accounts for the difference in partition functions and entropy between reactants and transition state. When the donor-acceptor interaction is sufficiently weak, $C^{\ddagger}$ can be neglected.[2,4]

Using the Weller relationship[1,2]

$$\Delta G^{\ddagger} = \frac{\Delta G}{2} + \left[\left(\frac{\Delta G}{2}\right)^2 + \left(\frac{\lambda}{4}\right)^2\right]^{1/2} \quad (7)$$

eq 3 can be transformed into

$$k_q = \frac{k_d}{1 + e^{\Delta G/RT} + \frac{k_{-d}}{k_{en}^0} e^{\{\Delta G/2 + [(\Delta G/2)^2 + (\lambda/4)^2]^{1/2}\}/RT}} \quad (8)$$

where the reorganization parameter λ associated with energy transfer is given by the following equation[5,6,12]

0002-7863/78/1500-7404$01.00/0