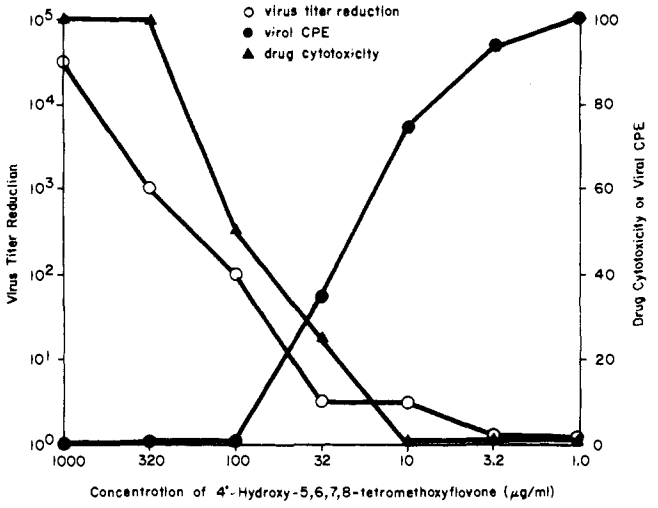


Figure 1.

Hydroxy-5,6,7,8-tetramethoxyflavone (**4**) had activity in repeated experiments against two of the three rhinoviruses tested. Against the type 13 virus, it had VR's ranging from 0.5 to 0.7. Against the type 56 virus a VR of 0.5 was obtained. Little activity was seen against the type 1A virus (VR 0.1-0.2). For comparative purposes, 2-methyl-4-[(5-methyl-5*H*-*as*-triazino[5,6-*b*]indol-3-yl)amino]-2-butanol (SK & F 30097), a compound known to possess activity against type 13 rhinovirus,[7] was tested in parallel experiments. This compound had VR's also ranging from 0.5 to 0.7 against all three rhinoviruses tested.

To further substantiate the antiviral activity seen, the effect of **4** on the quantity of infectious virus recoverable from treated KB cells was determined. In this experiment, 1000, 320, 100, 32, 10, 3.2, and 1.0 μg/ml of the compound were added to separate sets of cells 5 min before they were exposed to 100 cell culture infectious doses of type 13 rhinovirus. After a 3-day incubation at 33°, each group of cells was examined for inhibition of viral CPE as compared with untreated virus control cells. The cells were then disrupted by alternately freezing at −12° and thawing a total of three times. The supernatant fluid was then removed and varying dilutions of it added to a monolayer of KB cells which were in turn incubated for 3 days and examined for the presence of virus as evidenced by CPE. Reductions in virus titer ranging from 1 to 5 logs were seen in this experiment, with this titer reduction varying directly with the amount of compound originally added to the cells. The viral CPE inhibition and the titer reduction brought about by 4 is indicated in Figure 1.

Experimental Section

Infrared spectra were determined on a Perkin-Elmer 257 spectrophotometer (KBr), uv on a Cary 15 spectrophotometer, nmr on a Perkin-Elmer MR-20 using TMS as an internal standard, and mp on a Fisher-Johns block (corrected). C, H, and N were determined by M. H. W. Laboratories, Garden City, Mich., and Huffman Microanalytical Labs., Wheatridge, Colo.

2'-Hydroxy-3',4',5',6'-tetramethoxyacetophenone (1). Tangeretin† (4',5,6,7,8-pentamethoxyflavone) was degraded by treatment of 50 g in a refluxing mixture of 500 ml of EtOH and 500 ml of 40% KOH for 6 hr. The mixture was cooled and filtered and the filtrate was brought to pH 7-8 by addition of solid CO_2 (the pH adjustment can be facilitated by heating the basic solution during the addition of CO_2; this greatly reduces the foaming). The neutralized solution was extracted with Et_2O (3X). The combined Et_2O solutions were extracted 6 times with 5% NaOH. The combined basic washes were acidified with HCl and then extracted with Et_2O (3X). The Et_2O washes were combined, washed 2 times with saturated NaCl solution, and then dried over Na_2SO_4. The Et_2O was removed to yield 27 g (79%) of 1. The acetophenone (1) can be distilled under vacuum at 122–124° (0.75 mm).

4-Benzyloxy-2'-hydroxy-3',4',5',6'-tetramethoxychalcone (2). To a solution of 5.0 g (0.026 mmole) of *p*-benzyloxybenzaldehyde and 5.5 g (0.0212 mmole) of 1 in 100 ml of EtOH was added 10 ml of 50% KOH. The red solution stood at room temp for 24 hr and was then acidified to pH 3–4 with concd HCl. Water (125 ml) was added, and the solution extracted 3 times with Et_2O. The Et_2O was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was crystallized from MeOH to give 6.6 g (71%) of **2**, which melted at 95–98°. An analytical sample was crystallized from MeOH. *Anal.* ($C_{26}H_{26}O_7$) C, H.

4'-Benzyloxy-5,6,7,8-tetramethoxyflavone (3). The chalcone **2** was cyclized to the flavone by heating and stirring 5.0 g of **2** and 4.0 g of SeO_2 in 50 ml of *n*-amyl alcohol at 125° for 6 hr. The Se was removed by filtration of the mixture while still hot, and the filtrate was then cooled in the refrigerator giving crystalline 3. Recrystallization of the solid from MeOH gave 4.3 g (86%) of tan needles which melted at 145–146°. The reported mp is 145–146°.[5]

4'-Hydroxy-5,6,7,8-tetramethoxyflavone (4). Ease of removal of the benzyl group by hydrogenation was dependent on the purity of 3. A mixture of 2.2 g of 3 and 0.5 g of 5% Pd/C catalyst in 50 ml of AcOH was shaken at 1.6 atm of H_2 pressure (gauge) for 2.5 hr. The catalyst was removed by filtration and the filtrate evaporated. The residue was crystallized from MeOH to give 1.3 g (74%) of **4**. An analytical sample was crystallized from MeOH and melted at 200–202°. *Anal.* ($C_{19}H_{18}O_7$) C, H.

References

(1) R. W. Jones, M. G. Stout, H. Reich, and M. N. Huffman, *Cancer Chemother. Rep.*, 34, 19 (1964).
(2) A. Ben-Aziz, *Science*, **155**, 1026 (1967).
(3) L. Freedman and A. J. Merritt, *ibid.*, 139, 344 (1963).
(4) L. W. Wattenberg, M. A. Page, and J. L. Leong, *Cancer Res.*, **28**, 934 (1968).
(5) G. H. Stout and V. F. Stout, *Tetrahedron*, 14, 296 (1961).
(6) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1961).
(7) R. F. Haff, *Progr. Antimicrob. Anticancer Chemother., Proc. Int. Congr. Chemother., 6th*, **1969**, 818 (1970).

†Kindly supplied by Professor J. W. Kesterson, Citrus Experiment Station, Lake Alfred, Fla.

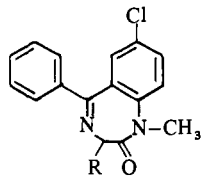
Mechanism of the Reaction of a 1,4-Benzodiazepine *N*-Oxide with Acetic Anhydride†

Neal Castagnoli, Jr.,* and Wolfgang Sadée

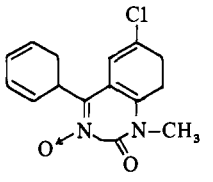
Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94122.
Received May 17, 1972

As part of our studies on the mechanism of microsomal mixed function oxygenations of nitrogen-containing compounds, we recently demonstrated that molecular oxygen and not water is the source of the C-3 oxygen atom found in the diazepam (**1**) biotransformation product, 3-hydroxydiazepam (**2**).[1] This carbinolimine metabolite is analogous to carbinolamines which have been proposed as unstable intermediates in microsomal oxidative N-dealkylations.[2] Evidence suggesting that N-oxidation species may be involved in the metabolic formation of carbon-oxygen bonds has been reviewed,[3] and several reports given at a recent symposium on the biological oxidation of nitrogen in organic molecules are concerned with the question of the possible intermediary role of nitrogen-oxygen bonds in microsomal C-oxidation.[4] The oxygen atom in metabolically formed N-O systems is reported to be O_2 derived.[5] There-

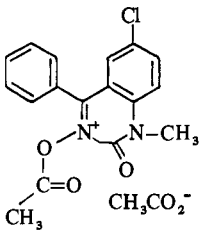
†Financial support from U. S. Public Health Service Grant GM-16496-04 is gratefully acknowledged.



1, R = H
2, R = OH
4, R = $OCCH_3$ (O')



3

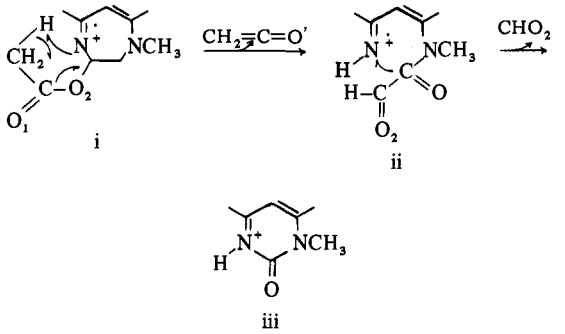


5

fore, should a nitrogen-oxygen bond participate in the metabolic transformation of **1** to **2**, an intramolecular nitrogen to carbon migration of the O_2-derived C-3 oxygen atom of **2** should be involved. In order to investigate the feasibility of such a migration, we have studied the nonenzymatic conversion of the nitrone 3 to the 3-acetoxy derivative **4**[6] with the aid of ^{18}O-enriched acetic anhydride.

A rational pathway for the formation of compound **4** proceeds by way of the acetoxyimmonium acetate **5**, either *via* an intramolecular migration of the *N*-acetoxy group or an intermolecular reaction involving the acetate anion generated from the acetic anhydride. With uniformly and highly ^{18}O-enriched acetic anhydride (95.52 atom %), it should be possible to distinguish between these two pathways. In the event of an exclusively intramolecular reaction, the *C*-acetoxy moiety should retain the ^{16}O of the original nitrone oxygen while any reaction involving participation of the acetate anion generated upon formation of adduct **5** should yield a product **4** in which both oxygen atoms of the acetoxy group are enriched with ^{18}O.

The mass spectrum of **4**[7] (in terms of the ^{35}Cl isotope only) shows a parent ion at *m/e* 342 and major fragments at *m/e* 300 and 271 (base peak) for which species i–iii, respectively, are proposed. This fragmentation pattern provides a means of estimating the ^{18}O enrichment of each



i ii iii

oxygen atom of **4** by determining the ratios of ion peak intensities at *m/e* to *m/e* + 2 for i, ii, and iii.

The mass spectrum of **4** obtained from the ^{18}O-enriched acetic anhydride reaction (Figure 1) revealed that essentially 100% of the N-^{16}O oxygen of **3** was retained in the acetoxy

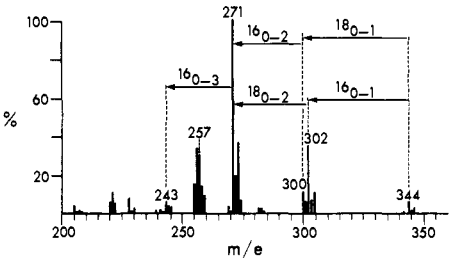
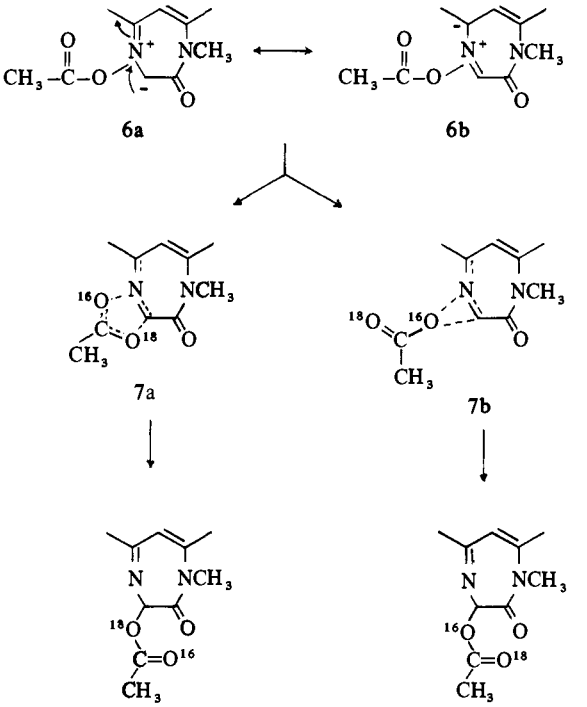


Figure 1. Low-resolution mass spectrum of 3-acetoxydiazepam (**4**) obtained from diazepam *N*-oxide (**3**) and 95.52 atom % ^{18}O-enriched acetic anhydride.

product. The peak height at *m/e* 342 corresponding to species $C_{18}H_{15}{}^{35}ClN_2{}^{16}O_3$ was approximately 5% of the major ion at *m/e* 344 and was consistent with the ^{16}O content of the starting acetic anhydride. Essentially no ion was observed at *m/e* 348 expected for species $C_{18}H_{15}{}^{37}ClN_2{}^{16}O^{18}O_2$. Furthermore, the ratio of peak intensities at *m/e* 344/346 corresponds to the $^{35}Cl/^{37}Cl$ natural abundance ratio confirming that the molecular ion, $C_{18}H_{15}ClN_2{}^{16}O_2{}^{18}O$, is the principal parent molecular species present. Based on the intensities of peaks at *m/e* 300 and 302 for ^{16}O-2 ii and ^{18}O-2 ii (Figure 1), it is apparent that the rearrangement of **5** to **4** proceeds with a scrambling of the N-^{16}O atom with 30% accounted for by the acyloxy oxygen (**4**, O-2) and the remaining 70% by the carbonyl oxygen (**4**, O-1). The peak intensities at *m/e* 43/45 for CH_3CO^+ resulting from an alternate fragmentation of i were found to be 70/30, confirming the above estimated $^{16}O/^{18}O$ ratio for the carbonyl oxygen. The ratio of the mass peaks at *m/e* 271/273 (iii) is the same as that found for the unlabeled compound, thus establishing that all of the ^{18}O was located in the acetoxy moiety.

A possible mechanism to account for these observations involves abstraction of H^+ from the initial acetoxyimmonium adduct **5** to yield the resonance-stabilized anion **6a** ↔ **6b** followed by a partial scrambling of the two oxygen atoms during product formation *via* either a 3-membered (**7a**) or 5-membered (**7b**) transition state. A similar mechanism has



6a **6b**

7a **7b**

been recently postulated[8] to account for the ^{18}O-labeling pattern, kinetics, and substituent effects observed in the rearrangement of α,*N*-diphenylnitrone to *N*-phenylacetanilide under the influence of acetic anhydride. However, chemically induced nuclear polarization studies on the acetic anhydride affected conversion of 4-picoline *N*-oxide to 4-acetoxymethylpyridine have demonstrated the existence of free radicals in this reaction.[9] A free radical mechanism for the conversion of **3** to **4** cannot be excluded at this time.

These results establish that in the system examined an intramolecular migration of oxygen from nitrogen to carbon can be affected *via* the activated *N*-acetoxyimmonium intermediate **5**. Recently the intramolecular rearrangement of the *N*-1-desmethyl analog of **3** to the corresponding 3-hydroxybenzodiazapine was reported to occur in the presence of a Lewis acid in acetonitrile,[10] thus providing additional evidence as to the facile nature of nitrogen to carbon migration of oxygen in the nitrone system. An enzymatic process can be envisioned in which an electron-deficient "active oxygen"[11] combines with the nucleophilic imino nitrogen of compound **1** to form a high energy enzyme–substrate complex analogous to **5**. Spontaneous or enzymatically facilitated intramolecular rearrangement of this immonium intermediate followed by regeneration of enzyme would yield the observed metabolite **2**. The chemical model examined in this work is consistent with such a pathway.

Experimental Section

Reaction of 1-Methyl-5-phenyl-7-chloro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-Oxide (3, Diazepam *N*-Oxide[11]) with ^{18}O-Enriched Acetic Anhydride. A solution of the *N*-oxide 3 (40 mg, 0.134 mmole) in acetic anhydride (0.2 ml, 95.52 atom % uniformly ^{18}O-enriched, Miles Lab.) was maintained at 80° for 1 hr under N_2. Upon cooling to 0° 1-methyl-3-[^{18}O]acetoxy-5-phenyl-7-chloro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4, 3-acetoxydiazepam,‡ 35 mg, 0.102 mmole, 76%) separated as colorless crystals. The material was dried over P_2O_5 *in vacuo* (90°): mp 261–263° (lit.[6] mp 262–263°).

Mass Spectroscopy. Mass spectra were obtained on an AEI MS 902 using a direct insertion probe. The electron-ionizing voltage was 70 eV at an ionizing current of 485 mA. The source temp was 210°.

References

(1) W. Sadée, W. Garland, and N. Castagnoli, Jr., *J. Med. Chem.*, 14, 643 (1971).
(2) J. R. Gillette, *Advan. Pharmacol.*, 4, 219 (1966); R. E. McMahon, H. W. Culp, and J. C. Occolowtz, *J. Amer. Chem. Soc.*, 91, 3389 (1969).
(3) M. H. Bickel, *Pharmacol. Rev.*, 21, 325 (1969).
(4) M. H. Bickel, *Xenobiotica*, 1, 313 (1971); A. H. Beckett, *ibid.*, 1, 487 (1971); P. Jenner, J. W. Gorrod, and A. H. Beckett, *ibid.*, 1, 497 (1971); M. H. Bickel, P. Willi, and P. L. Gigon, *ibid.*, 1, 533 (1971).
(5) J. R. Baker and S. Chaykin, *J. Biol. Chem.*, 237, 1309 (1962).
(6) S. C. Bell and S. J. Childress, *J. Org. Chem.*, 27, 1691 (1962).
(7) W. Sadée, *J. Med. Chem.*, 13, 475 (1970).
(8) S. Tamagaki, S. Kozuka, and S. Oae, *Tetrahedron*, 26, 1795 (1970).
(9) H. Iwamura, M. Iwamura, T. Nichida, and S. Sato, *J. Amer. Chem. Soc.*, 92, 7474 (1970).
(10) L. H. Schlager, *Tetrahedron Lett.*, 51, 4519 (1970).
(11) J. R. Gillette, *Biochem. Aspects Antimetab. Drug Hydroxylation, Fed. Eur. Biochem. Soc., Meet., 5th*, 1968, 109 (1969).

‡The authors wish to express their thanks to Dr. W. E. Scott of Hoffman-La Roche Inc., for supplying the diazepam derivatives used in this study.

2-Benzylaziridines. Cyclic Analogs of Amphetamines

Keith Brewster and Roger M. Pinder*

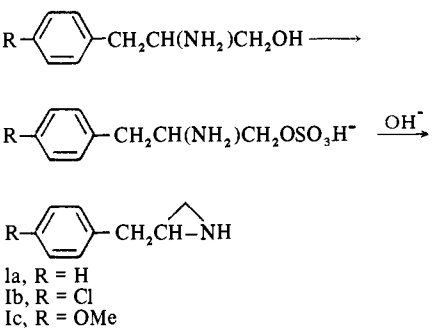
Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire, England. *Received February 22, 1972*

Aziridines exhibit a wide spectrum of biological properties and have found clinical application as antineoplastic agents.[1] In addition to interaction with cell constituents, the ability of aziridines to act as alkylating agents is reflected in drug–receptor interactions; for example, 2-haloalkylamines like dibenamine undergo cyclization *in vivo* to aziridinium ions prior to alkylation of the catecholamine α-receptor.[2] Our continuing interest in amphetamines and related compounds[3-5] prompted a study of the effects upon their biological activity of incorporation of part of the aminopropane side chain into an aziridine ring. 2-Benzylaziridine (Ia) has been described[6] but pharmacological data are not available. We now report the synthesis and pharmacology of this compound and its 4-chloro (Ib) and 4-methoxy (Ic) derivatives.

The classical Wenker synthesis of aziridines[1] (Scheme I) was satisfactory for Ia and Ib, but the sulfate ester of 4-methoxyphenylalaninol was obtained by dicyclohexylcarbodiimide coupling with H_2SO_4 in DMF[7] owing to the ease with which it underwent demethylation and subsequent decomposition under the very acidic conditions normally used. In our hands, a new synthesis of aziridines from vicinal amino alcohols using triphenylphosphine dibromide was singularly unsuccessful, though claimed yields are very poor for 1- and 3-unsubstituted aziridines.[8]

The three compounds had 0.1 the potency of amphetamine in reversing reserpine ptosis in mice,[4] but none of

Scheme I



Ia, R = H
Ib, R = Cl
Ic, R = OMe

them had any effect upon rabbit rectal temperature.[9] This relative lack of central stimulative actions is confirmed by their ineffectiveness in altering conditioned avoidance responses, in their inactivity in the Hall's open field test,[10] and in their lack of locomotor stimulation in mice;[5] indeed, Ia actually depressed spontaneous activity to a slight degree at 5 mg/kg. In the chloralosed cat, there were no effects *per se* at 5 mg/kg (iv) or upon the nictitating membrane contraction and responses to 5-hydroxytryptamine, noradrenaline, and isoprenaline.

It is likely that this lack of biological activity compared to amphetamine is due to the conformational restraint placed upon the molecule by incorporation of the amino function into the aziridine moiety, particularly since the analogous phenylcyclopropylamines exhibit considerable amphetamine-like activity.[11] However, it is possible that the aziridine ring