(7) R. A. Cummins and A. G. Moritz, *Aust. J. Chem.*, 18, 1107 (1965).
(8) A. A. Patchett, D. R. Hoff, and C. S. Rooney, U.S. Patent 3608087; *Chem. Abstr.*, **76**, 23951u (1972).
(9) D. J. Brown and R. F. Evans, *J. Chem. Soc.*, 4039 (1962).
(10) P. E. Fanta and E. A. Hedman, *J. Am. Chem. Soc.*, **78**, 1434 (1956).
(11) F. C. Schaefer and G. A. Peters, *J. Org. Chem.*, **26**, 412 (1961).
(12) A. Schwartz, *Circ. Res.*, **39**, 2 (1976).
(13) M. Hite, H. R. Skeggs, and H. E. Scribner, private communication.
(14) M. Cattell and H. Gold, *J. Pharmacol. Exp. Ther.*, **62**, 116 (1938).
(15) S. Garb, *J. Pharmacol. Exp. Ther.*, **101**, 317 (1951).
(16) R. Thorp and W. Cobbin, "Cardiac Stimulant Substances", Academic Press, New York, N.Y., 1967, p 24.
(17) K. Boniface, O. Brodie, and R. P. Walton, *Proc. Soc. Exp. Biol. Med.*, **84**, 263 (1953).
(18) D. E. Duggan and R. M. Noll, *Arch. Biochem. Biophys.*, **109**, 388 (1965).
(19) H. Matsui and A. Schwartz, *Biochim. Biophys. Acta*, **128**, 380 (1966).
(20) O. H. Lowry and J. Lopez, *J. Biol. Chem.*, **162**, 421 (1946).
(21) H. Schroeder and C. Grundmann, *J. Am. Chem. Soc.*, **78**, 2447 (1956).
(22) A. Albert and B. Paal, *Chem. Ind. (London)*, 874 (1974).
(23) G. Holan, E. L. Samuel, B. C. Ennis, and R. W. Hinde, *J. Chem. Soc. C*, 20 (1967).
(24) H. J. Roth, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, **292**, 194 (1959).
(25) R. O. Atkinson, *J. Chem. Soc.*, 1329 (1954).
(26) G. Shtacher, M. Erez, and S. Cohen, *J. Med. Chem.*, **16**, 516 (1973).

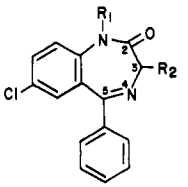
Pharmacology of Some Metabolites of Triazolam, Alprazolam, and Diazepam Prepared by a Simple, One-Step Oxidation of Benzodiazepines

Martin Gall,* B. V. Kamdar, and R. J. Collins

CNS Diseases Research, The Upjohn Company, Kalamazoo, Michigan 49001. *Received July 5, 1978*

A simple, one-step chemical oxidation of triazolam (**7**) to its 4-hydroxy analogue, **7a**, has been developed and applied to other triazolo- and imidazobenzodiazepines. The reaction may be used to convert diazepam to temazepam. 4-Hydroxytriazolo[4,3-*a*][1,4]benzodiazepines have low central nervous system sedative and anticonvulsant activity in sharp contrast to metabolites of diazepam which remain active. While **10**, an α-hydroxy metabolite of triazolam, retains much of the activity of **7**, **10a**, an α,4-dihydroxy metabolite of triazolam, is 250 times less potent than **7** on the nicotine-antagonism assay and over 300 times less potent on the traction assay.

Diazepam (**1**) is metabolized in man and the dog according to three major pathways: N-demethylation to demethyldiazepam (**2**), hydroxylation in the 3 position to temazepam (**3**), and N-demethylation plus 3-hydroxylation to oxazepam (**4**).[1] Other clinically useful benzodiazepines



1, $R_1 = CH_3$; $R_2 = H$ (diazepam)
2, $R_1 = H$; $R_2 = H$ (demethyldiazepam)
3, $R_1 = CH_3$; $R_2 = OH$ (temazepam)
4, $R_1 = H$; $R_2 = OH$ (oxazepam)
5, $R_1 = H$; $R_2 = H$ (4-*N*-oxide)
6, $R_1 = H$; $R = OCOCH_3$

are metabolized similarly in vivo.[2] In vitro hepatic microsomal preparations from mice enzymatically hydroxylate **1** and **2** at C-3 via the stereospecific removal of the pro-*S* hydrogen atom.[3] Moreover, various streptomyces stock cultures hydroxylate 1,4-benzodiazepin-2-ones in the 3 position; they do not produce 4-*N*-oxide derivatives (i.e., **5**) nor do they convert *N*-oxides to hydroxy derivatives (i.e., **5** ↛ **4**).[4,5] These results suggest that **4** is produced directly from **2**.

By contrast, the standard chemical synthesis of 3-hydroxybenzodiazepine derivatives such as **4** requires either converting the 4-*N*-oxide, **5**, to the acetate, **6**, by brief heating in acetic anhydride, followed by careful hydrolysis with sodium hydroxide,[6] or treating **5** with Lewis acids in nitrile solvents to obtain **4** directly.[7] Similar methods have been used to prepare the 4-hydroxytriazolo and -imidazo analogues, **8a**[10a,c] and **13a**.[11] Both methods require the 4-*N*-oxide derivative, which is not always conveniently prepared from the parent benzodiazepine.[7b]

In this paper we describe a direct, one-step oxidation procedure which cleanly converts diazepam (**1**), triazolobenzodiazepines such as triazolam (**7**) and alprazolam (**8**), and imidazo analogue **13** to the corresponding hydroxy derivatives (**3**, **7a**, **8a**, and **13**). We also describe the animal pharmacology of the triazolobenzodiazepine metabolites whose structure–activity relationship differs considerably from the parent diazepam metabolites.

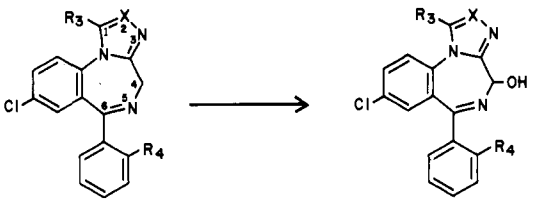
Chemistry. When oxygen was bubbled through a cold tetrahydrofuran (THF) solution of the green-black lithium anion generated from **13** and lithium diisopropylamide (procedure A), alcohol **13a** was isolated in only 11% yield.[8a] The major byproducts were partially characterized dimers of **13** (see Experimental Section).

In contrast, oxidation at −20 °C of the green-black potassium anion obtained from **13** and a slight excess of potassium *tert*-butoxide in a mixture of *tert*-butyl alcohol–DMF–THF–DME–$(EtO)_3P$ produced **13a** in 50% yield, unaccompanied by dimeric side products (procedure B).[8b] Keeping the reaction temperature cold and quenching the reaction mixture in ice-cold 5% acetic acid were essential; the 1,2-dimethoxyethane (DME) cosolvent could be replaced by THF. This procedure for oxidation of triazolam (**7**) to **7a** produced crude yields as high as 95%. Representative yields (not maximized) for the oxidation of various benzodiazepines by procedure B are summarized in Table I.

Oxidation of alcohol **11**, itself a metabolite of alprazolam, deserves further comment. With **11** as the substrate, procedure B yielded only the starting material. Since the characteristic green-black color associated with benzodiazepine anions was not observed, we conclude that the requisite anion did not form. However, the anion was

0022-2623/78/1821-1290$01.00/0

Table I. Oxidation of [1,4]Benzodiazepines



R_3	R_4	X	starting material (mmol)	KO-*t*-Bu, mmol	product	% yield[a,b] (crude)	mp,[b] °C (solvent)	formula	analyses[c]
CH_3	Cl	N	7 (45)	70	7a	74 (95)	292-293.5[j] ($MeOH-CH_2Cl_2$)	$C_{17}H_{12}Cl_2N_4O$	C, H, N, Cl
CH_3	H	N	8 (80)	120	8a	39 (58)	235-238[k,n] ($MeOH-CHCl_3$)	$C_{17}H_{13}ClN_4O$	C, H, N, Cl
CH_2NMe_2	H	N	9 (10)	83	9a	15[d]	232-234[l] (MeOH-EtOAc)	$C_{19}H_{18}ClN_5O$	C, H, N, Cl
CH_2OH	Cl	N	10 (5.5)	11	10a	53	272-274 ($MeOH-CHCl_3$)	$C_{17}H_{12}Cl_2N_4O_2 \cdot 0.5H_2O$	C, H, N, Cl
CH_2OH	H	N	11 (2.5)	16.5	11a	19[e-g]	241-242 (MeOH-EtOAc)	$C_{17}H_{13}N_4ClO_2 \cdot 0.5CH_3OH$	C, H, N, Cl
CH_2OS-*t*-Bu	H	N	12[g] (14.8)	50	12a	12 (18)[h]	229-230 (MeOH-EtOAc)	$C_{23}H_{27}ClN_4O_2Si$	C, H, N, Cl
H	H	CH	13 (10)	15	13a	50	185-187[m] (EtOAc)	$C_{17}H_{12}ClN_3O$	C, H, N, Cl
			1 (10)	15	3	33 (90)	119-122[i] (CH_2Cl_2-Et_2O)	$C_{16}H_{13}ClN_2O_2$	C, H, N, Cl

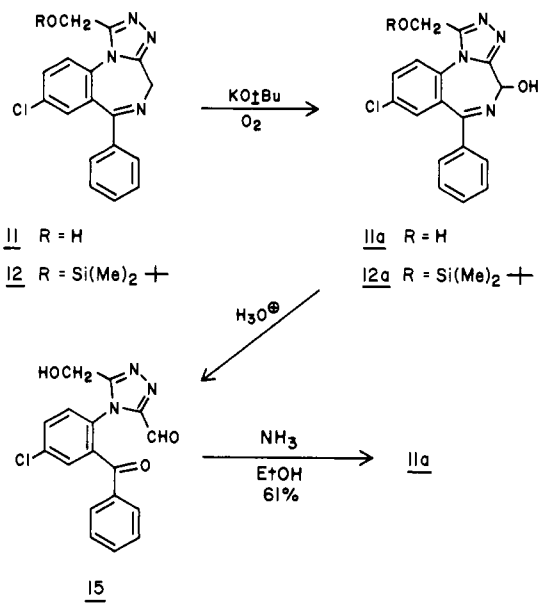
[a] The values refer to isolated crystallized yields. Values in parentheses refer to crude yields of product isolated without chromatography. [b] Procedure B was used for these reactions; melting points refer to analytically pure samples. [c] Satisfactory analyses were obtained for the elements indicated. [d] Reproducing the yield of this reaction was difficult. The yield is not maximized. [e] Procedure B yielded no 11a. See procedure C. [f] A second component present in this reaction mixture could not be characterized further. [g] See Experimental Section. [h] The yields reported are from procedure B. Typically 12a was accompanied by about 5-10% 11a and occasionally some 11. When the reaction was cooled below -45 °C, 12a could be obtained in about 90% crude yield, 30% after recrystallization. [i] Lit.[6c] mp 119-121 °C. [j] Mp[10d] 294-295 °C (CH_2Cl_2-MeOH). [k] Mp[10a] 242 °C dec ($CHCl_3$-EtOH as an EtOH solvate). [l] Mp[10d] 237-239 °C (CH_2Cl_2-EtOAc). [m] Lit.[11b] mp 192-194 °C (cyclohexane-benzene). [n] Lit.[10c] mp 245.5 °C (DMF-H_2O).

generated when the concentration of *tert*-butyl alcohol was reduced and the number of equivalents of base was increased. In the best example of the direct oxidation of **11**, 6.6 equiv of potassium *tert*-butoxide was used to produce a 19% yield of **11a** following oxidation, chromatography, and crystallization (see procedure C).

The reactions were usually quenched by pouring the solution into cold 5% acetic acid and adding 1 N sulfuric acid. When the quench was inverted, that is, when the acid was added to the cold reaction mixture of **11**, a solid of mp 184–185 °C, having a $P(OEt)_2$ group and analyzing for $C_{21}H_{23}ClN_3O_6P$ (**14**) (see Experimental Section), was obtained; **11a** was not detected. When the reaction was quenched normally, but the products were kept in the acid for a prolonged period of time (i.e., >10 min), substantial quantities of hydroxyketoaldehyde **15** were isolated (see Scheme I). Because of these difficulties and because separating product **11a** from aldehyde **15** was surprisingly difficult, the silyl-protected alcohol, **12**, was prepared. (Because of its poor solubility in cold DMF, **12** was dissolved in a minimal volume of CH_2Cl_2 and diluted to the desired volume with DMF.) At the usual reaction temperature, approximately -20 °C, the protecting group was partially removed and varying quantities of **11**, **11a**, and **12a** were isolated from the reaction mixture. However, when the reaction temperature was kept below -45 °C, **12a** could be isolated in 90% crude yield directly from the aqueous workup; recrystallizing the product resulted in a great loss in yield (see Table I, footnotes *f* and *g*). Since we were unable to remove the protecting group without hydrolyzing the benzodiazepine ring,[9] we converted **12a** to aldehyde **15** and then, by analogy with a procedure previously used to prepare **7a**–**9a**,[10a,d] we re-formed the benzodiazepine ring in EtOH–NH_3 to give **11a** in the yields shown in Scheme I.[10a]

The crude yield of temazepam (**3**) from diazepam (**1**) was >90%, but crystallizing **3** in the presence of excess $(EtO)_3P$ was difficult and the yield of purified **3** was reduced. Using

Scheme I



procedure B we could not successfully metalate and oxidize demethyldiazepam (**2**) to oxazepam (**4**). **2** was the only benzodiazepine we tried which we could not successfully metalate. Apparently the secondary amide must first be protected in order to achieve metalation. However, we anticipate that our procedure will be useful for the direct oxidation of all benzodiazepines which can be metalated at the methylene position.

Results

4-Hydroxytriazolam (**7a**) had a very low order of central nervous system activity. For example, in the dish and nicotine-antagonism assays, **7a** was 250 and >2000 times, respectively, less potent than triazolam (**7**) (see Table II). Similarly, 4-hydroxyalprazolam (**8a**) was 40 and 200 times,

Table II. Comparative Pharmacological Data[a] for Hydroxylated Metabolites of Benzodiazepines

					antagonism						
no.	Tr-50	Ch-50	D-50	Ped In	nicotine	pentylenetetrazole	thiosemicarbazide	strychnine	max electric shock	EtOH	hypoxic stress
7	0.30	0.08	0.03	0.03	0.004	0.009	0.020	0.22	30	0.70	0.035
7a	>100	45	8.0	11.0	10.0	14	11.2	>80	>100	>80	>100
8	0.60	0.09	0.15	0.23	0.02	0.13	0.16	0.30	25	0.18	0.02
8a	23	6.3	6.3	20	4.0	0.7	5.0	25	>50	10.0	6.2
9	5.6	3.6	1.5	2.8	0.20	0.80	0.15	>200	100	2.2	1.9
9a	22	4.0	5.0	22	2.2	4.0	0.88	>40	>100	>20	25
10	0.6	0.14	0.03	0.17	0.010	0.018	0.014	0.50	89	0.4	0.50
10a	>100	2.2	5.0	44	1.0	32	0.39	>80	>100	50	30.0
11	8.0	0.63	2.0	1.56	0.08	0.22	0.08	9.9	50	1.8	0.02
11a	>100	>80	56	>80	6.2	40	8.9	>80	>100	>80	>50
13	16	3.6	5.0	4.0	1.0	2.2	1.6	7.0	32	3.6	25
13a	32	>25	6.0	7.0	4.0	6.3	6.3	>80	63	7.9	60
1	4.0	1.2	0.25	0.70	0.10	0.80	0.40	4.0	25	1.0	0.20
3	4.0	0.45	0.36	0.70	0.063	0.30	0.30	2.8	10.0	0.32	0.2
2	7.0	0.70	0.50	0.90	0.30	1.6	1.8	20	>100	1.8	0.7
4	13.0	1.8	1.1	2.0	0.14	0.70	0.90	9.0	79.0	1.8	12.5

[a] Biological data expressed as ED_{50} values in mg/kg. These ED_{50} values have a 95% confidence interval of about ±100%.

respectively, less potent than alprazolam (**8**) in the same two assays. Somewhat less marked potency differences were noted for the 4-hydroxytriazole derivatives **9a–11a** vs. **9–11**.

The differences in potency between the parent and 4-hydroxylated triazolobenzodiazepines contrasted sharply with the activity seen for both diazepam (**1**) and demethyldiazepam (**2**) and their respective 3-hydroxylated derivatives, temazepam (**3**) and oxazepam (**4**). In fact, temazepam and oxazepam were *more* potent than diazepam and demethyldiazepam, respectively, in the anticonvulsant assays. However, while temazepam was approximately equipotent to diazepam in the traction, dish, and pedestal assays, oxazepam was one-half as potent as demethyldiazepam in these assays.

In contrast to the large potency differences seen between parent and 4-hydroxytriazolobenzodiazepines, hydroxyimidazobenzodiazepine **13a** was only one-half to one-quarter as potent as the parent **13** (cf. traction and nicotine assays). However, imidazole **13** was 50 times less potent than triazole **8** in the nicotine assay.

Discussion

1-Demethylation and 3-hydroxylation of diazepam produce several metabolites which retain much of the parent compound's activity. Temazepam (**3**), demethyldiazepam (**2**), and oxazepam (**4**) are quite active and unquestionably contribute to diazepam's biologic effect.[1a,b]

Triazolobenzodiazepines such as triazolam (**7**) and probably alprazolam (**8**) are primarily metabolized by α- or 4-hydroxylation or both.[19,20] α-Hydroxylation of these triazolo compounds reduces activity slightly (**7** vs. **10** and **8** vs. **11**). However, hydroxylation of triazolo- and imidazobenzodiazepines at position 4 reduces potency considerably (see Results and Table II). Potency loss may be greater for the more potent compounds (e.g., **7** vs. **7a** and **8** vs. **8a**) than for the less potent compounds in the series (**9** vs. **9a** and **13** vs. **13a**). Hydroxylation at both the α and 4 position produces compounds with little activity (**10a** and **11a**).

In summary, the 4-OH metabolites of triazolobenzodiazepines probably do not contribute to the biological effect of the parent compound. While the α-OH metabolites of triazolobenzodiazepines do retain considerable biological activity, their contribution to the therapeutic effect of the parent drug is likely quite low since the plasma levels of **10** in man are much less than those of the parent compound, **7**.[21] Moreover, **10** does not accumulate with chronic dosing. In contrast, a series of biologically active compounds are derived from the metabolism of diazepam (**1**), and the plasma levels of these metabolites increase with chronic dosing.[21b]

Experimental Section

Chemistry. Melting points measured with a Thomas-Hoover capillary melting point apparatus are uncorrected. The structures of all compounds were supported by IR, UV, NMR, and mass spectral data. IR spectra were determined in Nujol with a Perkin-Elmer Model 421 recording spectrophotometer. NMR spectra were recorded on a Varian Model A-60D or a Varian Model XL-100; chemical shifts were recorded in δ parts per million downfield from tetramethylsilane. Mass spectra were obtained with a Varian MAT CH7 or LKB. Starting materials **7**, **8**, and **13** have been described.[10,11] Compounds **10** and **11** were prepared by the reaction of 7-chloro-1,3-dihydro-5-(*o*-chlorophenyl)-2*H*-1,4-benzodiazepine-2-thione and 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepine-2-thione,[22] respectively, with hydroxyacetic acid hydrazide in refluxing *n*-BuOH.[10d] Compound **9** was prepared[10a] by the reaction of 8-chloro-1-(chloromethyl)-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepine with dimethylamine.

Preparation of 12. A stirred solution of **11** (6.0 g, 0.018 mol) in 25 mL of DMF was treated with an ice-cold solution of *t*-BuSi(Me)$_2$Cl (6.75 g, 0.045 mol) and imidazole (6.0 g, 0.090 mol) in 20 mL of DMF. After the resulting suspension was stirred overnight at room temperature, the reaction mixture was poured into cold water and extracted with 2 × 200 mL portions of chloroform. The chloroform extract was washed with water and brine, then dried, and concentrated in vacuo to 8 g (98%) of white solid, mp 216–218 °C. Crystallization from ethyl acetate yielded 6.5 g of white needles, mp 217–219 °C.[12] Anal. ($C_{23}H_{27}ClN_4SiO$) C, H, N, Cl.

Procedure A. **13** (5.86 g, 0.020 mol), dissolved in 20 mL of freshly distilled 1,2-dimethoxyethane (DME), was slowly added to a solution of lithium diisopropylamide generated from 15.0 mL of 1.6 M MeLi (0.024 mol) and 3.62 mL (0.030 mol) of diisopropylamine in 10 mL of DME at −78 °C. Dry oxygen was blown over the surface of the reaction mixture at −70 to −50 °C. Oxygen was then bubbled through the reaction mixture at 0–20 °C for 1.5 h. Water (10 mL) was added and the reaction mixture was stirred overnight. The solvents were removed in vacuo and the products chromatographed over silica gel by eluting with 3% MeOH–97% $CHCl_3$ mixtures. Fractions[13] 37–52 were combined, concentrated, and crystallized from $CHCl_3$–MeOH mixtures to afford 1.20 g of fine yellow-orange needles (**16**), mp 348–350 °C dec. A weak molecular ion at *m/e* 585 (587) with fragment ions at *m/e* 482 and 293 (295) indicated that this material was a dimer of **13**. The analytical data fit. Anal. ($C_{34}H_{22}Cl_2N_6$, M_r 585.48)

C, H, N, Cl. Obtaining this material solvent free was difficult.

Fractions 104–106 contained an oil which crystallized from acetone to yield 130 mg of fine yellow needles, mp 205–210 °C, followed by solidification, with remelting at 300 °C. This material was not further characterized.

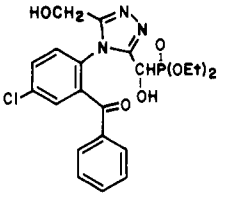
Fractions 169–191 contained a yellow oil which crystallized from ethyl acetate–hexane mixtures to yield 1.05 g of amorphous powder, mp 140–148 °C (bubbles). This sample was recrystallized from ethyl acetate to yield 0.68 g (11%) of **13a** as needles, mp 185–187 °C. Anal. ($C_{17}H_{12}ClN_3O$) C, H, N, Cl.

Finally, fractions 205–277 were combined, concentrated, and crystallized from ethyl acetate to afford 0.61 g of flowers, mp 308–311 °C dec. This was recrystallized from $CHCl_3$–MeOH mixtures to afford 0.47 g of flowers, mp 326–329 °C. After drying at 100 °C in a vacuum oven this sample discolored from 230 to 240 °C and melted with decomposition above 300 °C. It was not further characterized.

Procedure B. A solution of 1.5 M KO-*t*-Bu (15 mmol) in 10 mL of THF and 65 mL of distilled *t*-BuOH in a two-neck flask with rubber septum was treated with a solution of $(EtO)_3P$ (2.67 g, 16 mmol) in 20 mL of DME in a nitrogen atmosphere. The resulting stirred mixture was cooled to −20 °C with a CCl_4–dry ice bath and treated with a solution of the starting material (10 mmol) in 25 mL of DMF. The green-black solution of the anion of the starting material was stirred for 0.5 h at −20 °C at which time a slow stream of dry oxygen was bubbled through the reaction mixture until the anion color was quenched (usually within 0.75–1.5 h). The reaction mixture was quenched in 100 mL of ice-cold aqueous 10% acetic acid, treated with 100 mL of 1 N H_2SO_4, and neutralized in the cold with a 1 N Na_2CO_3 solution. Alcohols **7a** and **8a** could be filtered directly from this aqueous medium. When the analogous reaction was performed with **12** as the substrate, at a temperature below −45 °C, **12a** could also be filtered directly from the aqueous quench. For the other products, it was necessary to extract the aqueous layer with chloroform–methanol mixtures. The organic layer was washed with water and brine, then dried over Na_2SO_4, filtered, and concentrated in vacuo to an oil. The oil was chromatographed over silica gel by eluting with 3%/97% to 10%/90% methanol–chloroform mixtures. The products[12] were crystallized in the yields shown in Table I. Compounds **7a** and **8a** were identical with authentic samples prepared from the benzodiazepine *N*-oxides.[10a] (See the footnotes to Table I.)

Procedure C. A solution of 1.5 M KO-*t*-Bu (3.0 mmol) in 12 mL of THF and 2.5 mL of *t*-BuOH at −78 °C was treated with **11** (0.83 g, 2.5 mmol) dissolved in 12.5 mL of DMF. A green-black color formed initially but then disappeared. The addition of 1.5 mL of KO-*t*-Bu (2.25 mmol) in THF regenerated the dark anion color. The green-black color was retained after the addition of 3.0 mL of *t*-BuOH but faded to a light yellow color following the addition of 2.0 mL more of *t*-BuOH. The addition of 2.0 mL of DMF did not produce any change, but the further addition of 2.0 mL of 1.5 M KO-*t*-Bu (3.0 mmol, total of 8.25 mmol) did regenerate the anion. While the temperature was maintained at −78 °C, dry oxygen was bubbled into the reaction mixture. Five minutes after the start of the oxidation $(EtO)_3P$ (0.44 g, 2.65 mmol) was added. While the anion color disappeared after 20 min, an aliquot worked up as described in procedure B contained a considerable amount of starting material:[14a] R_f (**11**) 0.12, (**11a**) 0.22. The reaction mixture was therefore treated with an additional 7.0 mL of DMF and 5.5 mL of 1.5 M KO-*t*-Bu (8.25 mmol). The green-black color of the anion appeared immediately but disappeared within 2 min. After an additional 5-min stirring period, the entire reaction mixture was worked up as described above. While TLC indicated that all of the starting material had reacted, the spot corresponding to the product now consisted of two zones readily separated by silica gel chromatography by eluting with 90% acetone–10% chloroform mixtures. This yielded 500 mg of an incompletely characterized oil related to **15** (vide infra) (R_f[14b] 0.53) and 240 mg of crude **11a** (R_f[14b] 0.26), which crystallized from methanol–ethyl acetate mixtures to give 160 mg of flowers, mp 237–241 °C dec.

In a separate experiment using 10 mmol of **11**, 45 mmol of KO-*t*-Bu, and 32 mmol of $(EtO)_3P$ in 50 mL of THF, 65 mL of *t*-BuOH, and 85 mL of DMF, at a temperature of −20 °C, the reaction mixture was quenched *by adding to it* 100 mL of 10% acetic acid, followed by 100 mL of 1 N H_2SO_4. This produced **14** with an R_f[14a] similar to that of **11a** (1.73 g, 36.2%): mp 184–186 °C. Anal. ($C_{21}H_{23}ClN_3O_6P$, M_r 479.84) C, H, N, Cl. **14** has tentatively been assigned the following structure.[12]



14

Finally, an experiment in which the temperature of the normal acid quench rose above 15 °C before complete neutralization produced a 3% yield of **11a**. The major product of the reaction, hydroxyaldehyde **15**, was isolated in 34% yield. A sample crystallized from methanol–ethyl acetate mixtures as flowers: mp 125–134 °C (foams). Anal. ($C_{17}H_{12}ClN_3O_3 \cdot CH_3OH$) C, H, Cl, N. The sample decomposed in a vacuum oven at 90 °C.

Aldehyde **15** (0.67 g, 1.98 mmol), suspended in 30 ml of absolute EtOH, was stirred 10 min and then treated with 20 mL of a saturated ethanolic ammonia solution.[10a] Within 5 min all suspended solids had dissolved. After the mixture was stirred overnight at room temperature, the solvent was removed in vacuo and the resulting foam was crystallized from methanol–ethyl acetate–hexane mixtures to furnish 425 mg (61%) of **11a** as flowers in two crops: mp 240–244 °C (decomposes with bubbling) (see Table I). Anal. ($C_{17}H_{12}N_3O_3Cl \cdot CH_3OH$) C, H, Cl, N.

Pharmacology. Methods. Male, albino CF-1 mice weighing 18–22 g were used in all studies. Test compounds were suspended in 0.27% aqueous methylcellulose solution and administered intraperitoneally to groups of six mice. Each compound was tested at four or more dose levels spaced at a 0.3 log interval to calculate the ED_{50} as described by Spearman and Karber.[15] Procedures for measuring the effect of the test compounds on simple reflexes such as the traction (Tr-50), chimney (Ch-50), dish (D-50), and pedestal (Ped In) tests have been described.[16] Likwise, procedures for measuring antagonism of seizures induced by nicotine salicylate, pentylenetetrazole, thiosemicarbazide, strychnine, and maximal electroshock and those for measuring depressant activity as indicated by potentiation of ethanol have been described.[17] The hypoxic stress test, which correlates with anxiolytic activity, has also been reported previously.[18]

Acknowledgment. The authors are indebted to Cathy White and Rollie Russell for capable laboratory assistance, Drs. A. D. Rudzik, P. F. VonVoigtlander, and R. A. Lahti for helpful discussions, and Helen Branch and Suzanne Moyer for the preparation of this manuscript. We are happy to acknowledge the work of Paul Marlatt and associates which resulted in the preparation of 100 g each of **8a**, **11**, and **11a**.

References and Notes

(1) (a) M. A. Schwartz, B. A. Koechlin, E. Postma, S. Palmer, and G Krol, *J. Pharmacol. Exp. Ther.*, **149**, 423 (1965); (b) L. O. Randall and B. Kappell in "The Benzodiazepines", S. Garattini, E. Mussini, and L. O. Randall, Eds., Raven Press, New York, N.Y., 1973, p 27.

(2) (a) M. A. Schwartz in ref 1b, p 53; (b) G. K. Woo, S. J. Kolii, and M. A. Schwartz, *Pharmacologist*, **19**, 164 (1977).

(3) A. Corbella et al., *J. Chem. Soc., Chem. Commun.*, 721 (1973).

(4) F. Kishimoto, K. Suzucki, S. Ogino, and H. Yamamoto, *Agric. Biol. Chem.*, **39**, 953 (1975).

(5) W. Sedee, W. Garland, and N. Castangnoli, *J. Med. Chem.*, **14**, 643 (1971).

(6) (a) S. C. Bell and S. J. Childress, *J. Org. Chem.*, **27**, 1691 (1962); (b) S. C. Bell, C. Gochman, and S. J. Childress, *ibid.*, **28**, 3010 (1963); (c) S. C. Bell et al., *J. Med. Chem.*, 11, 457 (1968); (d) S. C. Bell, R. J. McCaully, and S. J. Childress, *J. Org. Chem.*, **33**, 216 (1968).

(7) (a) L. H. Schlager, *Tetrahedron Lett.*, 4510 (1970); (b) A. Walser, L. E. Benjamin, Sr., T. Flynn, C. Mason, R.

Schwartz, and R. I. Fryer, *J. Org. Chem.*, **43**, 936 (1978).
(8) (a) Our failure to add a reducing agent to the reaction mixture may have contributed to the low overall yield. (b) Based on a procedure described by G. Buchi, P. Kulsa, and R. L. Rosati, *J. Am. Chem. Soc.*, **90**, 2448 (1968); G. Buchi, P. Kulsa, K. Ogasawara, and R. L. Rosati, *ibid.*, **92**, 999 (1970); and E. J. Barley, D. H. R. Barton, J. Elks, and J. F. Templeton, *J. Chem. Soc.*, 1578 (1962).
(9) The use of $(Bu)_4N^+F^-$ in THF led to extensive decomposition of **12a** to unidentified products.
(10) (a) J. B. Hester, Jr., personal communication; (b) J. B. Hester, Jr., D. J. Duchamp, and C. G. Chidester, *Tetrahedron Lett.*, 1609 (1971); J. B. Hester, Jr., A. D. Rudzik, and B. V. Kamdar, *J. Med. Chem.*, 14, 1078 (1971); (c) K. Meguro and Y. Kuwada, U.S. Patent 3 907 829 (1975); (d) J. B. Hester, Jr., and G. N. Evenson, unpublished results.
(11) J. B. Maffrand, G. Ferrand, and F. Eloy, *Tetrahedron Lett.*, 3449 (1973); *Eur. J. Med. Chem.-Chim. Ther.*, **9**, 539 (1974).
(12) The IR, UV, NMR, and mass spectral data supported the proposed structure.
(13) Twenty-milliliter fractions were collected.
(14) (a) The TLC analysis was performed on silica gel G by eluting with 10% methanol–90% chloroform mixtures; (b) the analysis was performed on silica gel G by eluting with a mixture of 90% acetone–10% chloroform.
(15) D. J. Finney, "Statistical Methods in Biological Assay", Hafner Publishing Co., New York, N.Y., 1952.
(16) G. A. Youngdale, D. G. Auger, W. C. Anthony, J. P. DaVanzo, M. E. Greig, R. V. Heinzelman, H. H. Keasling, and J. Szmuszkovicz, *J. Med. Chem.*, 7, 415 (1964).
(17) A. D. Rudzik, J. B. Hester, Jr., A. Tang, R. N. Straw, and W. Friis in ref 1b, p 285.
(18) R. B. Moffett, B. V. Kamdar, and P. F. VonVoigtlander, *J. Med. Chem.*, 19, 192 (1976).
(19) F. S. Eberts and L. Baczynskyj, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **32** (3), 747 (1973).
(20) F. S. Eberts, *Pharmacologist*, 16, 196 (1974).
(21) (a) F. S. Eberts, personal communication; (b) L. Hillestad, T. Hansen, H. Melsom, and A. Drivenes, *Clin. Pharmacol. Ther.*, 16, 485 (1974).
(22) G. A. Archer and L. H. Sternbach, *J. Org. Chem.*, **29**, 231 (1964).

Absolute Configuration of (+)-5-(3-Hydroxyphenyl)-5-phenylhydantoin, the Major Metabolite of 5,5-Diphenylhydantoin in the Dog

James H. Maguire, Thomas C. Butler, and Kenneth H. Dudley*

Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514.
Received June 5, 1978

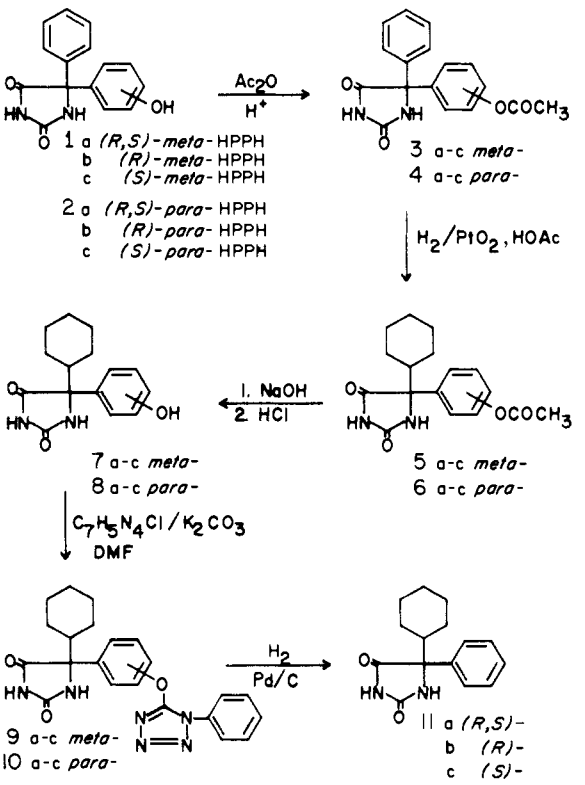
5-(3-Hydroxyphenyl)-5-phenylhydantoin (*m*-HPPH) has been resolved by crystallization of the brucine salts. The (+) enantiomer has been converted to (−)-5-cyclohexyl-5-phenylhydantoin, a compound previously demonstrated to have the *R* configuration.[3] The *R* configuration can accordingly be assigned to (+)-*m*-HPPH, the principal metabolite of 5,5-diphenylhydantoin (phenytoin) in the dog.

The metabolism of 5,5-diphenylhydantoin (phenytoin, DPH) has been studied in several species of animals. Reports published through the year 1971 have been reviewed by Chang and Glazko.[1] In all species examined with the exception of the dog, the principal metabolite is 5-(4-hydroxyphenyl)-5-phenylhydantoin (*p*-HPPH), which appears in urine largely conjugated with glucuronic acid. As DPH is a prochiral molecule, the two phenyl rings are spatially distinguishable and are not equivalent in enzymatic reactions. Introduction of a hydroxyl group in one of the phenyl rings creates a chiral center at carbon-5 of the hydantoin ring, and stereoselectivity of hydroxylation is reflected in optical activity of phenolic metabolites. In *p*-HPPH released by β-glucuronidase from its conjugate in human urine there was about a 10:1 preponderance of the levorotatory isomer relative to the dextrorotatory.[2] In enzymatically released *p*-HPPH from dog urine, the preponderance of the levorotatory isomer was about 2:1.

The amount of 5-(3-hydroxyphenyl)-5-phenylhydantoin (*m*-HPPH) in human urine is so small that isolation has not as yet been accomplished, and its stereoisomeric constitution is unknown. In the dog, on the other hand, *m*-HPPH is the major metabolite of DPH. Enzymatically released *m*-HPPH from dog urine was dextrorotatory in ethanol and alkaline aqueous solution and had the properties of an essentially pure optical isomer.[2]

Interest in the stereoselectivity and regioselectivity of the metabolism of the prochiral DPH molecule has directed attention to the absolute configurations of the phenolic metabolites. The assignment of the *S* configuration to (−)-*p*-HPPH (**2c** of Scheme I), the major metabolite of DPH in man, was accomplished by application of the "rule of shift" (Poupaert et al.[3]) and confirmed through X-ray crystallographic examination of the camphorsulfonate of (+)-*p*-HPPH (**2b**) (Koch et al.[4]). By conversion of **2b** to (−)-5-cyclohexyl-5-phenylhydantoin (**11b**), it was possible to make an assignment of the absolute configuration of the latter compound.[3]

Scheme I



0022-2623/78/1821-1294$01.00/0