Receptor Site Labeling through Functional Groups. Barbital and Amphetamine Derivatives

TETSUO MIYADERA,[1] EDWARD M. KOSOWER,*[2]

Department of Chemistry, State University of New York, Stony Brook, New York 11790

AND NECHAMA S. KOSOWER[3]

Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461

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A probe for a drug receptor site can be assembled from a fragment (D) derived from a drug of proven biological activity, a connection of variable length and character (C_n), and a moiety (Y) capable of reactivity toward a limited set of functional groups (F) at or near the receptor site. Covalent bond formation between Y and F should result in labeling of the receptor site, making possible its isolation. If the biological effects of the D-C_n-YF combination are similar to those of D, long-term activity could result. A number of derivatives of barbital and *d*-amphetamine with C_n = C_2, C_3, C_5, or C_2OC_2 and Y = 2,3-dimethylmaleimidyl are described, together with the results of a limited series of biological tests (rats, firefly larvae). Many of the compds exhibit activity, indicating that the C_nY substituent on D does not abolish the interaction of D with the receptor sites (which may not be the same as those involved with the action of the drug D). The duration of action of the derivatives is not prolonged, implying that an SH group is not readily accessible at or near the receptor sites.

The character of biological receptors for many drugs and transmitter molecules has remained elusive in spite of the urgent need for information which might permit the design of suitable substrates for these sites.[4] The methodology[5,6] developed for the active sites of enzymes (*e.g.*, diisopropyl phosphorofluoridate for α-chymotrypsin) has not been easy to extend to receptors for drugs because the evaluation of success with the latter usually requires a biological test. We have now formulated an approach related to that of Baker[5] which should be applicable to a variety of receptor sites.

In the past few decades, many useful drugs have been developed (*e.g.*, "sulfas," diuretics, steroids, psychopharmacological agents) through the strategy called "molecular modification" from discoveries made by chance or in the course of other work.[7,8] It must be admitted that such molecular sculpture is made difficult by the absence of any clear data about the nature and dimensions of the space to be filled. In order to probe the nature of the receptor site (the volume the drug occupies in order to cause its biological action), we must label that site. To design an appropriate label, we must begin with structures of known biological activity, drugs which exhibit considerable affinity for the sites of interest. To fix the drug at the site, we must substitute into the drug molecule a reactive fragment capable of combining with a suitable functional group at or near the receptor site. Since the functional group may be located some distance from the region in which the drug fragment is combined, a connection is provided, and the length of that connection varied. The drug fragment is designated as D, the connection as C_n, the reactive fragment as Y, and the functional group at or near the receptor site as F. Choosing a suitable structure for D–C_n–Y then involves the resolution of 3 problems.

1. Does the attachment of C_nY to D abolish the biological activity inherent in D?
2. Does the group Y have sufficient reactivity to bind to F, yet not so much as to react with other similar groups during passage through biological barriers?
3. Is the nature and length of the connection C_n appropriate for the binding of D–C_n–Y to F?

Our choice of drug was guided by the necessity for a highly visible biological effect for the evaluation of the activity of the modified drug. For this reason, we chose barbiturates, sleep-inducers, and amphetamines, CNS-activity-inducers. Picking an SH-reactive group as the group Y was done on two grounds, first, that there are now a number of indications that SH groups are located near receptor sites for ACh[9-11] and that SH groups are somehow involved in determining the electrophysiological characteristics of the neuromuscular junction,[12] and second, that SH groups are among the most reactive functional groups available in proteins, allowing the choice of (relatively) unreactive groups for Y.

For this initial investigation, it was more important to demonstrate biological activity than to choose the ideal structure for Y. The high reactivity of *N*-ethylmaleimide toward SH groups (eq 1) and the low, but discernible reactivity of *N*-ethyl-2,3-dimethylmaleimide toward thiols (eq 2) caused us to utilize these groups for our receptor site probes. It was most convenient to measure reactivity with glutathione (GSH) (or cysteine) in neutral aq sol for two reasons: first, GSH (usual intracellular concn 10^{-4} to 10^{-3} *M*) represents a natural hazard to the passage of drug from the region of administration to the receptor site and second, through suitable corrections for different thiol pK_a's and the assumption that the thiol would have a concn

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(3) Career Development Awardee of the National Institutes of Health.

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(8) E. J. Ariëns, *Fortschr. Arzneimittelforsch.*, **10**, 429 (1966).

(9) A. Karlin and E. Bartels, *Biochim. Biophys. Acta*, **126**, 525 (1966).

(10) A. Karlin and M. Winnik, *Proc. Nat. Acad. Sci. U. S.*, **60**, 668 (1968).

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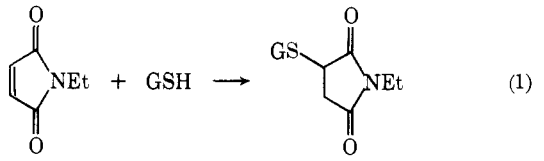
(12) R. Werman, P. L. Carlen, M. Kushnir, and E. M. Kosower, *Nature (London)*, in press (1971).



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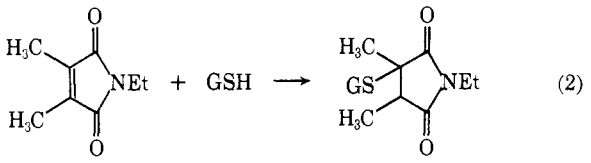

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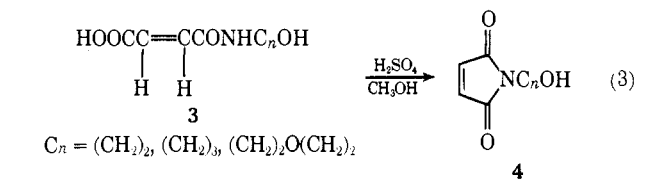
of 10 M with respect to any SH-reactive group bound to the receptor site, the rate of binding of Y to F could be predicted. In fact, one of the powerful advantages of our particular approach is that the binding group Y can be evaluated by kinetic measurements on simple model compds before complex syntheses are undertaken and before biological tests are run. Once the ground rules for a particular drug type are established (the group C_nY does not greatly alter biological activity, an SH-binding group is at or near the receptor site), the Y group can probably be varied considerably in structure to change the strength of binding of Y to F and the rate at which such binding is achieved.

Synthesis.—The prepn of the maleimide alcohols (**4**, precursor of **7**) could be accomplished *via* the maleamic acids.[13] The latter were isolated in a number of cases (eq 3,5). The presence of a group reactive toward



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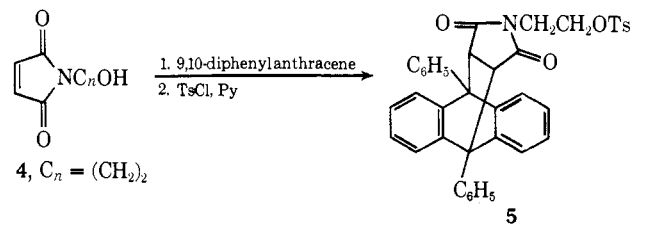

4, $C_n = (CH_2)_2$

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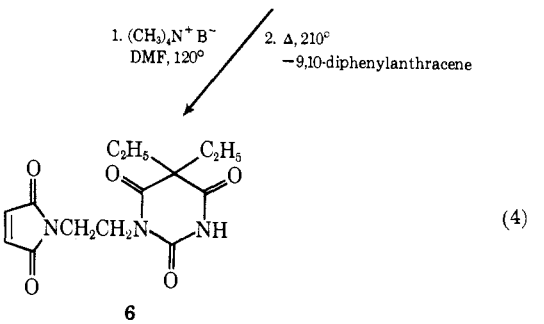

(4)

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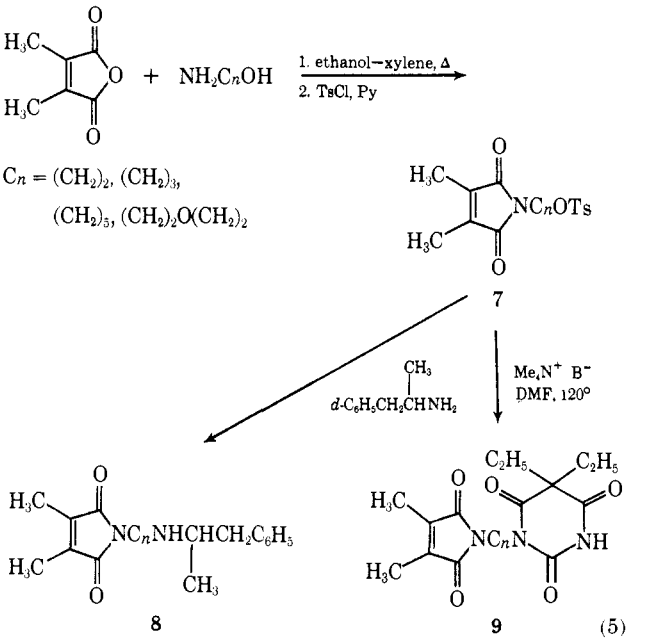
nucleophiles in the unsubstituted maleimides (**4**) required a protecting group for the nucleophilic substitution by the barbiturate anion. After attempts to utilize anthracene, 9-phenylanthracene, and cyclopentadiene were made, 9,10-diphenylanthracene was found to be satisfactory[14] (eq 4) for the successful



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completion of the retro Diels–Alder reaction. Displacement of the tosylate group by barbiturate was carried out in the aprotic dipolar solvent, DMF, at 120°. (N-Alkyl derivatives of barbituric acids are generally produced by forming the ring from the alkylamines. A few alkylations have been reported in EtOH.[15]) To achieve a reasonable reaction rate, the Me_4N salt of barbital was used since the Na salt was not very soluble in hot DMF. Bis-alkylated products (bis-**9**) were isolated in all cases but were not usually examined further. The amphetamine derivatives were produced from the tosylates by reaction with (+)-amphetamine in DMF.

The structures of all isolated products were confirmed by nmr and ir spectra. The properties of the maleimides, maleimidyl tosylates, maleimide barbitals, and maleimidylamphetamines are tabulated in Tables I–IV, resp. Several succinimidyl derivatives were prepared by way of the tosylate (eq 6), and these are listed in Table V.

Rate Studies.—The rate of reaction of GSH with N-ethylmaleimide (**1**) has been measured;[16,17] the rate constant at pH 7.3 is approximately 2300 M^{-1} sec^{-1} at 25° (eq 1). We have examined the rate of reaction of GSH with N-ethyl-2,3-dimethylmaleimide (**2**) in aq soln, pH 7.2, and have found the initial rate constant for the reaction to be 7.3×10^{-3} M^{-1} sec^{-1}. From these data, we may estimate certain biological properties of the maleimides and their drug derivatives.

(a) Thiol Barrier to Arrival at Receptor Site.—The most reactive thiol present in reasonable concns in

(13) M. Masamori, M. Yamada, I. Takase, K. Hayashi, Y. Hashimoto, and Y. Komiya, *Yuki Gosei Kagaku Kyokai Shi*, **23**, 166 (1965); *Chem. Abstr.*, **63**, 489 (1965).

(14) W. E. Bachmann and M. C. Kloetzel, *J. Amer. Chem. Soc.*, **60**, 481 (1938).

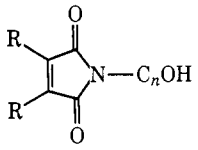
(15) I. Gazauzov and D. Dalev, *Farmatsiya (Sofia)*, **16**, 12 (1966); *Chem. Abstr.*, **66**, 65443 (1967).

(16) G. Gorin, P. A. Martic, and G. Doughty, *Arch. Biochem. Biophys.*, **115**, 593 (1966).

(17) F. Schneider and H. Wenck, *Hoppe-Seyler's Z. Physiol. Chem.*, **350**, 1521 (1969).

TABLE I

PROPERTIES OF *N*-ω-HYDROXYALKYLMALEIMIDES[a]



R	C_n[b]	Mp or bp (mm), °C	Precursor	Yield, %
H	$(CH_2)_3$	39–40	MAA deriv[b,c]	45
H	$(CH_2)_2O(CH_2)_2$	130 (0.005)	MAA deriv[d]	41
CH_3	$(CH_2)_2$	97–98 (0.05)	DMA	94
CH_3	$(CH_2)_3$	110 (0.15)	DMA	88
CH_3	$(CH_2)_5$	122 (0.05)	DMA	85
CH_3	$(CH_2)_2O(CH_2)_2$	137 (0.3)	DMA	93

[a] Nmr and ir data were in accord with these structures. [b] Ref 13 includes data for compd with $C_n = (CH_2)_2$. [c] MAA, maleamic acid; DMA, dimethylmaleic anhydride.

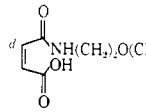
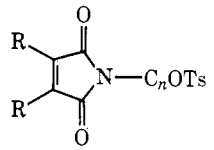
[d]  , mp 67–68°, was prepd according to the synth method previously described (ref 13).

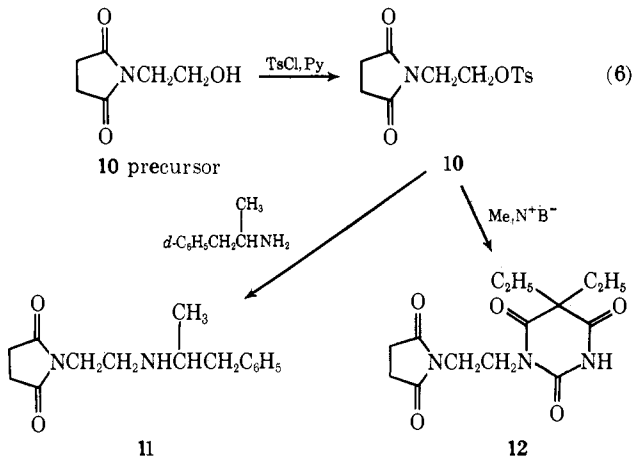
TABLE II

PROPERTIES OF *N*-ω-TOSYLOXYMALEIMIDES[a]



R	C_n[b]	Mp, °C	Yield, %
H	$(CH_2)_2$	122–123[c]	66
CH_3	$(CH_2)_2$	138–139[c]	70
CH_3	$(CH_2)_3$	57–58[d]	56

[a] Nmr and ir data were in accord with the structures. [b] The other two tosylates ($C_n = (CH_2)_5$ and $(CH_2)_2O(CH_2)_2$) were obtd as viscous oils which were used without further purification for the subsequent reaction. [c] From PhH. [d] From Et_2O.



(6)

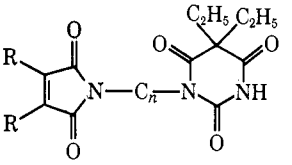
biological systems is GSH.[18] From the equation for the half-life of a bimolecular reaction, we can estimate the time required for disappearance of half of the initial dose. [A dose of 100 μmoles/kg = 10^{-4} *M* drug; (GSH) = 10^{-4} *M* (or more) (eq 7)].

$$t_{1/2} = 1/(k_2 \times c_0) \quad (c_0 = (\text{GSH}) = (\text{D})) \qquad (7)$$

(18) E. M. Kosower and N. S. Kosower, *Nature (London)*, **224**, 117 (1969), and ref cited therein.

TABLE III

PROPERTIES OF *N*-MALEIMIDYLBARBITALS[a]

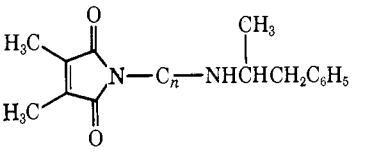


R	C_n	Mp, °C	Yield, %
H	$(CH_2)_2$	162–163	21
CH_3	$(CH_2)_2$	139–140	28
CH_3	$(CH_2)_3$	112–113	26
CH_3	$(CH_2)_5$	81–82	20
CH_3	$(CH_2)_2O(CH_2)_2$	86–87	24

[a] Nmr and ir data were in accord with the structures.

TABLE IV

PROPERTIES OF *N*-2,3-DIMETHYLMALEIMIDYLAMPHETAMINES[a]

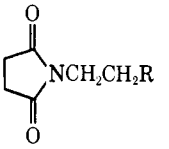


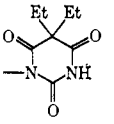
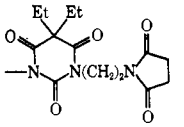
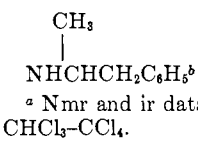
C_n	Bp (mm),[b] °C	Yield, %
$(CH_2)_2$	150 (0.908)	71
$(CH_2)_3$	170 (0.05)	65
$(CH_2)_5$	170 (0.003)	66
$(CH_2)_2O(CH_2)_2$	180 (0.03)	72

[a] Nmr and ir data were in accord with the structures. [b] Bath temp.

TABLE V

PROPERTIES OF SUCCINIMIDE DERIVATIVES[a]



R	Mp or bp, (mm), °C	Yield, %
OH	61–62[b]	88
OTs	103–104	83
	146–147	26
	175–176	50
	170–80 (bath temp) (0.006 mm)	68

[a] Nmr and ir data were in accord with the structures. [b] From $CHCl_3$–CCl_4.

For *N*-ethylmaleimide, the half-life is ~5 sec, while for the 2,3-dimethyl analog, the half-life is ~10^6 sec. Thus derivatives of **1** would be "detoxified" by reaction with GSH before any physiological effects would be noted. In fact, MalBarb (**6**) behaved exactly like NEM (**1**) (Table VI). Derivatives of **2**, however, could easily reach equilibrium with the receptor sites.

TABLE VI
EFFECTS OF INTRACEREBRAL BARBITURATES AND RELATED COMPOUNDS

	Mortality, %[a] — Dose injected, μmoles[b]					
Compd	6	4	2	1	0.3	0[b]
NEM (1)		100	100	67	33	0
Barbital[c]		0[d]	0[d]			
MalBarb (6)		100	100			
DEM (2)	0[e]	0[e]	0[d]			
THE[d]	0[d]	0[d]	0[d]			

[a] Death usually followed progressive weakness and slow deterioration within 10–24 hr, although several died within 1 hr. [b] Total dose injected: μmoles of substance in 50% PG-0.075 *M* NaCl (PG = propylene glycol); control injections: 0.15 ml of 50% PG-0.075 *M* NaCl. [c] 5,5-Diethylbarbituric acid. [d] No observable behavioral effect. Minor effects would not be noticed if they occurred during the period of recovery from Et_2O anesthesia (2–3 min). [e] Transient weakness noted, from which the animals recovered within 20 min.

(b) Rate of Blocking of Receptor Site.—The equilibrium concn of a drug in the biological compartment containing the receptor site is very difficult to estimate, and changes with time due to redistribution, excretion, and metabolism. In addition, the binding constants for the drug to the receptor site are not known. The rate of reaction of the *bound* drug with an SH group at the receptor site can be estimated by assuming that the drug is present in 10 *M* concn and that the thiol concentration is also 10 *M*. (These are the relative concns for next-neighbor groups in pure organic molecules, and the choice of concns is equivalent to the assumption that the reactive group and the SH group are in proximity to one another.) Let X be the covalently combined receptor site-drug, then the rate of formation (in moles/l. sec) of X is given by eq 8 for the 2,3-dimethylmaleimido

$$\frac{dX}{dt} = 7.3 \times 10^{-3}\ M^{-1}\ \text{sec}^{-1}\ (10\ M)(10\ M) = 7.3 \times 10^{-1}\ \text{moles/sec} \quad (8)$$

derivative. To a first approximation, the concn of occupied receptor sites (RD) can be estimated by assuming a reasonable binding constant (10^3) and some number for the total concn of receptor sites (R) (10^{-4} *M*) (eq 9). Given these assumptions, we find that

$$(\text{RD}) = K(\text{R})(\text{D}) = 10^3(10^{-8})(10^{-5}) = 10^{-10}\ M \text{ drug concn} \quad (9)$$

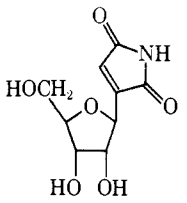
only 1% of the binding sites would be occupied and the net rate of formation of X should be $5 \times 10^{-1} \times 10^{-2}$ or 5×10^{-3} moles/l. sec. If our boundary conditions are reasonable, this means that a drug containing a 2,3-dimethylmaleimido group should be bound to an SH group near the receptor site. Our biological results do not suggest long-term binding of any agent at neuroactive receptor sites, and we must conclude that no SH group is present near these sites.

Discussion

Except for 1-Me derivatives of barbiturates, relatively little effort has been expended in the direction of 1-substituted variants.[19] *N*-Substitution in the amphetamine series gives rise to compds which are active, although not necessarily in the same way as the parent compd.[20] Our first task was to ascertain whether or not biological activity could be retained after transforming the drug molecules into the desired derivatives. In this respect, we appeared to be successful in both the barbiturate and amphetamine series, since most of the derivatives did give rise to neurological effects. The length of the chain (connection, C_n) does make a difference but our tests were too limited for definite conclusions. One interesting result is the behavior of the **9** barbiturate (n = 2-O-2) as a fast-acting compound of short duration, suggesting that other barbiturates with the same substituent might be worth examination.

According to our measurement of the rate constant for the reaction for GSH and *N*-ethyl-2,3-dimethylmaleimide, we should expect our drug derivatives to be bound to a site containing an SH group. The failure to observe prolonged biological effects of any kind in any of the tests suggests that there is no SH group near the receptor sites for amphetamines or barbiturates.

It must not be thought that maleimide derivatives are only *in vitro* reagents. The antibiotic, showdomycin (**14**),[21] recently synthesized,[22] is proof that such compds can be found in biological systems. We surmise that the mechanism of action of **14** involves addition of an SH group to the double bond. Another natural SH reagent is α-L-glutaminyl-3,4-benzoquinone (**15**).[23] It is likely that additional natural and synthetic thiol-reactive compds (see ref 18) will be discovered in the future; drugs with SH-reactive substituents are complementary to these agents.



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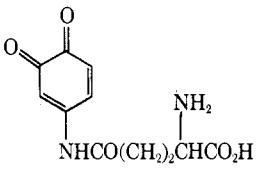

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Experimental Section

All nmr spectra were measured with an A-60 spectrometer, and ir spectra with Perkin-Elmer Models 700 or 257 spectrophotometers.

***N*-(2-Hydroxyethyl)maleimide (4, C_n = $(CH_2)_2$).**—*N*-(2-Hydroxyethyl)malea mic acid[13] (30.0 g, 0.189 mole) was treated with H_2SO_4 (0.9 ml) in MeOH (300 ml) at 25° for 6 days, and, after neutralization and removal of ppt and solvent, yielded a colorless oil, bp 130° (0.005 mm) (13.6 g, 51%), which crystd on standing, mp 70–71°.[13] Other unsubstituted maleimides were prepd in the same way, and their properties are listed in Table I (**4**, C_n = $(CH_2)_3$, $CH_2CH_2OCH_2CH_2$).

***N*-(2-Hydroxyethyl)-2,3-dimethylmaleimide (7 Precursor, C_n = $(CH_2)_2$).**—2-Aminoethanol (1.90 g, 31 mmoles) was added with stirring to 2,3-dimethylmaleic anhydride (3.78 g, 30 mmoles) (Aldrich Chem. Co.) in EtOH (20 ml). *m*-Xylene (45 ml) was added, the mixt was brought to gentle reflux, and the C_6H_6 was distd off. Xylene was removed under vacuum, and the residue was distd to yield an oil: bp 97–98° (0.05 mm), 4.76 g, 94%. Other 2,3-dimethylmaleimides were prepd in the same way (**7** precursor, C_n = $(CH_2)_3$, $(CH_2)_5$, $CH_2CH_2OCH_2CH_2$), and are

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(21) H. Nishimura, M. Mayama, Y. Komatsu, K. Kato, N. Shimaoka, and Y. Tanaka, *J. Antibiot., Ser. A*, **17**, 148 (1964).

(22) L. Kalvoda, J. Farkas, and F. Sorm, *Tetrahedron Lett.*, 2297 (1970).

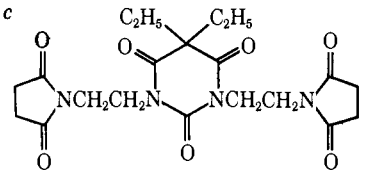
(23) R. F. Weaver, K. V. Rajagopalan, P. Handler, P. Jeffs, W. L. Byrne, and D. Rosenthal, *Proc. Nat. Acad. Sci. U. S.*, **67**, 1050 (1970).

TABLE VII

EFFECTS OF INTRAPERITONEAL BARBITURATES AND RELATED COMPOUNDS[a]

Compd	Sleeps[b]	Duration, min	Other effects
Barbital	+ (20–30)	180–240	
DEM (2)	–		Very slight weakness
DEMB-2 (9, n = 2)	–		Some weakness; shivering (60)
DEMB-3 (9, n = 3)	–		
DEMB-5 (9, n = 5)	–		
DEMB-202 (9, n = 2-O-2)	+ (10)	60–80	
SB 1-1 (12)	?	120–180	Somnolent; weak
SB 2-1[c]	–	20–30	Groggy; weak

[a] A single dose between 70 and 80 μmoles in 50% propylene glycol–0.075 M NaCl was injected ip. [b] Number in parentheses indicates the interval in minutes between injection and sleep.



listed in Table I. The succinimide derivative (**10** precursor) was prepd in the same way.

9,10-Diphenyl-9,10-ethano-9,10-dihydroanthracene-[*N*-(2-hydroxyethyl)]-11,12-dicarboximide (5 Precursor) and Tosylate (5).—*N*-(2-Hydroxyethyl)maleimide (1.06 g, 7.52 mmoles) and 9,10-diphenylanthracene (2.48 g, 7.52 mmoles) were refluxed in PhMe (10 ml) for 5 days. The ppt was collected, washed with PhMe, and recrystd from PhMe to yield colorless crystals, mp 237–238° (2.10 g, 59%). The compd (4.00 g, 8.50 mmoles), suspended in pyridine (35 ml), was treated with *p*-TsCl (5.6 g, 29.4 mmoles) for 3 hr at 0–5°, the reaction mixt was poured into ice water, and the ppt was collected, washed with H_2O, dried, and recrystd from EtOAc to yield a white solid, mp 248–249° (2.83 g, 53%). (**5**) The properties are listed: **5** precursor in Table I, **5** in Table II. Other tosylates (**7**, **10**) were prepared in a similar way, except that a reaction time of 2 hr was used.

Tetramethylammonium 5,5-Diethylbarbiturate (TMA$^+$B$^-$).—$Me_4N^+OH^-\cdot 5H_2O$ (45.3 g, 0.25 mole) was added to 5,5-diethylbarbituric acid (46.0 g, 0.25 moles) in MeOH (200 ml), the soln was evapd to dryness, and the residue was recrystd from MeOH to yield colorless crystals: mp 227–228° dec; 49.0 g, 76.2%; nmr (DMSO-d_6), triplet 9.38 (CH_3CH_2), quartet 8.42 (CH_3CH_2), singlet 6.84.

1-(2-Maleimidylethyl)-5,5-diethylbarbituric Acid (6).—TMA$^+$B$^-$ (1.20 g, 4.67 mmoles) was dissolved in DMF (25 ml), tosylate **5** (1.60 g, 2.56 mmoles) was added, the soln was heated at 120° for 2 hr, and the mixt was poured into ice water. The ppt was filtered off, dried (1.51 g), and pyrolyzed with stirring at 210° under reduced pressure (0.05 mm). The sublimate (1.27 g) was collected and chromatographed on silica gel (Fisher, 28–200 mesh). Elution with CH_2Cl_2 yielded 9,10-diphenylanthracene (0.721 g) and with CH_2Cl_2–$(CH_3)_2CO$ (9:1) gave **6** as colorless crystals (0.418 g) which were recrystd from $CHCl_3$ to give colorless plates, mp 162–163° (166 mg, 21%). Properties are recorded in Table I.

1-[2-(2,3-Dimethylmaleimidyl)ethyl]-5,5-diethylbarbituric Acid [9, C_n = $(CH_2)_2$].—TMA$^+$B$^-$ (1.54 g, 6.00 mmoles) was dissolved in DMF (25 ml) at 120° with stirring, the **7**-C_2 tosylate (**7**, C_n = CH_2CH_2) (0.969 g, 3.00 mmoles) was added, and the mixt was stirred 2 hr at 120°. The reaction mixt was poured into ice water and extd with $CHCl_3$, the ext was washed with H_2O and dried (Na_2SO_4), the solvent was evapd, and the oily residue was chromatographed on neutral alumina (Woelm, activity grade IV). Elution with PhH–$CHCl_3$ (4:1) gave bis-**9** (170 mg) (structure—2:1 ratio of ethylene and 5,5-diethyl groups by nmr in CCl_4), and then 1-methyl-5,5-diethylbarbituric acid (small amts, identical with authentic). Elution with $CHCl_3$ gave a cryst substance (450 mg) which was recrystd from EtOAc to yield colorless crystals, mp 139–140° (280 mg, 28%).

TABLE VIII

EFFECT OF INTRAPERITONEAL AMPHETAMINE AND DERIVATIVES[a]

Compd	Effect	Duration of effect, hr
d-Amphetamine	Excitation (shivering, running)	3[b]
SA-2 (11)	Sight weakness	1
DMA-2 (8, n = 2)	Convulsions; severe weakness alternating with moderate excitation	10–15 mn 3–4
DMA-202 (8, n = 2-O-2)	Slight shivering and consulsive behavior; mild weakness	1–2
DMA-5 (8, n = 5)	Weakness	1–2

[a] A dose of approximately 30 μmoles in 50% propylene glycol–0.05 M phosphate, pH 7.4, was injected ip. [b] *Cf.*, K. W. Miller, J. J. Freeman, J. V. Dingell, and F. Sulser, *Experientia*, **26**, 863 (1970).

TABLE IX

EFFECT OF *d*-AMPHETAMINE AND DERIVATIVES ON LIGHT EMISSION FROM FIREFLY LARVAE[a]

Compd	Light output, mV[b,c]	Time to max output, min
d-Amphetamine	3.7	1.7
DMA-2 (8, n = 2)	2.7	35
DMA-3 (8, n = 3)	2.8	9
DMA-5 (8, n = 5)	2.3	9
SA-2 (11)	2.3	35

[a] According to procedures described in A. D. Carlson, *J. Exp. Biol.*, **48**, 381 (1968); **49**, 195 (1968). [b] Amplified output of phototube in mV. [c] Electrical stimulation was usually ineffective in producing a burst of light after long exposure to DMA derivs.

The nmr spectrum was in excellent accord with the desired product (Table III). Except for details of chromatogr and crystn, other **9** derivatives were prepd in a similar way (listed in Table III).

***N*-Ethyl-2,3-dimethylmaleimide (2).**—2,3-Dimethylmaleic anhydride (1.26 g) in C_6H_6 (7 ml) was mixed with $EtNH_2$ (0.46 g) in *p*-xylene (30 ml). After slowly distg off the solvent, the residue was distd and treated with excess $EtNH_2$ in petr ether, and the filtrate was distd to give a colorless oil: bp 105° (15 mm); 0.6 g, 40%; nmr (CCl_4) τ 8.86 (CH_3, t, J = 7.3 cps), 6.53 (CH_2, 9., J = 7.3 cps), 8.09 (2 CH_3, s).

***N*-Ethyl-3,4,5,6-tetrahydrophthalimide** was prepd in the same way, bp 145° (11 mm), in 77% yield. The oil crystd, and recrystn from petr ether gave colorless crystals: mp 46–47°; nmr (CCl_4) τ 8.86 (CH_3, t, J = 7.3 cps), 6.54 (CH_2, q, J = 7.3 cps), 8.20 (4 H, m), 7.71 (4 H, m).

***N*-2-Succinimidylethyl-2-amino-1-phenylpropane (11).**—The tosylate **10** (1.49 g, 5.00 mmoles) and *d*-amphetamine (2.02 g, 15.0 mmoles) in DMF (10 ml) were stirred at 120° for 2 hr, the reaction mixt was poured into H_2O and extd with $CHCl_3$, the org layer was washed with H_2O, dried (Na_2SO_4), and evapd to give a residue which was distd to yield the product as a viscous oil, bp 170–180° (bath) (0.006 mm) (0.890 g, 68%). Other amphetamine derivatives were prepd from **7** tosylates in an analogous way (Table IV).

Biological Tests.—Sprague–Dawley female rats (150–160 g) were used. Solns (or suspensions) of the barbiturate derivatives were prepd by soln in redistd propylene glycol, followed by diln with an equal vol of isotonic NaCl (final soln 50% PG–0.075 M NaCl). Solns (or dispersions) of the amphetamine derivatives were made in a similar way to give a final prepn of 50% PG-0.05 M phosphate, pH 7.4. Between 1 and 3 animals were used for each of the tests, the purpose of which was to indicate whether or not the compds possessed any biological activity at all.

Intracerebral injections (ic) were carried out with 0.15 ml and are summarized in Table VI. Ip injections (ip) were made with 2.0 ml and are listed in Tables VII and VIII.

Firefly larvae tests were carried out by Professor A. D. Carlson and Miss Nancy Littell, Department of Biological Sciences, State University of New York, Stony Brook, N. Y. Light output from emitting cells was monitored by a phototube during exposure to a soln of test compd[24] (Table IX).

Rate Studies.—Soln of *N*-ethyl-2,3-dimethylmaleimide in 0.5 *M* phosphate buffer (stable for >24 hr) were degassed by alternate exposure to vacuum and N_2 (6 cycles). The buffer was then poured into a side arm contg the appropriate amt of GSH. After shaking briefly to ensure soln, the cell contg the reaction soln was placed in the light path of a Cary Model 14 spectrophotometer. Reaction was followed first at 3050 A, then by complete spectroscopic curves. Optical densities at different times at 305.0 nm were measured and the rate constants computed with the aid of a second-order rate equation and a General Electric Computer. A complete description of the kinetic properties of *N*-alkylmaleimides will appear in a future article.

(24) A. D. Carlson, *J. Exp. Biol.*, **48**, 381 (1968); **49**, 195 (1968).

Tetracyclic Quinazolinone Derivatives

GOETZ E. HARDTMANN,* BRUNO S. HUEGI,

Department of Chemical Research

JOHN H. GOGERTY, LOUIS C. IORIO, AND H. WILLIAM BARNES

Pharmacology Section, Department of Biological Research, Sandoz Pharmaceuticals, Division of Sandoz-Wander, Inc., Hanover, New Jersey 07936

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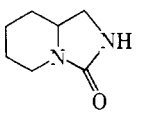
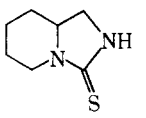
The preparation of 1,6,7,8,9,9a-hexahydro-4-methylthio-2*H*-pyrido[1,2-*c*]pyrimidine and 1,5,6,7,8,8a-hexahydro-3-methylthioimidazo[1,5-*a*]pyridine and their reactions with isatoic anhydride and with anthranilic acid are described. The pharmacology of the reaction products is discussed.

Recently Ziegler and coworkers[1] investigated the reaction of isatoic anhydride with 2-methylmercaptoimidazoline. We now would like to describe the reaction of 2 novel bicyclic mercaptomethylureas with a variety of isatoic anhydrides and anthranilic acids and discuss the pharmacological properties of the reaction products.

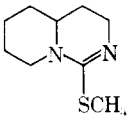
The first saturated imidazo[1,5-*a*]pyridine, namely, the urea **1**, was reported by Winterfeld and Schueler.[2] When we allowed pipecolylamine[3] to react with CS_2[4] the thiourea **2** was obtained, which on reaction with MeI yielded **3**. Analogously the homolog **4** was prepared from 2-(2-aminoethyl)piperidine.[5]



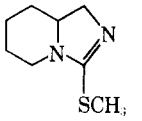
1



2

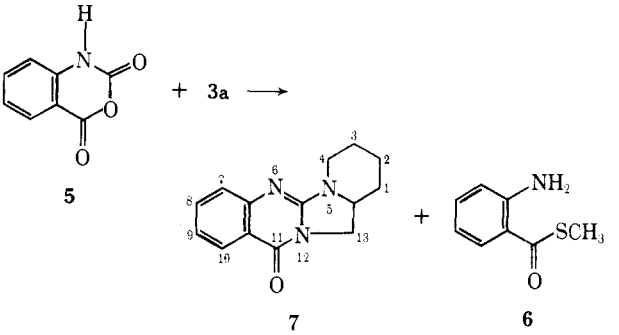


4, ·HI
4a



3, ·HI
3a

When equimolar amounts of the free base **3a** and of isatoic anhydride (**5**) were allowed to react at 100° in dioxane, 2 new products were formed. One was identified as thioanthranilic acid *S*-Me ester (**6**)[6] and the other was the expected tetracyclic material **7**. Compd



5 **7** **6**

4a did not react with **5** under similar conditions, but the desired **8** (see Table I) was obtained when **5** was replaced by anthranilic acid. In the same manner, 4,5-dimethoxyanthranilic acid and several other anthranilic acids (see Experimental Section and Table I) could be treated with **3a** and **4a**.

Pharmacology.—In the course of preliminary investigations on the pharmacological activities of a series of tetracyclic quinazolinone derivatives, it was noted that these substances provided a profile of CNS-depressant activity not unlike that obtained with standard sedative–hypnotics. Each of the present series of compds was submitted to a battery of behavioral and drug-interaction tests in mice, with selected compds being further investigated in behavioral tests in squirrel monkeys. The results with the test compds were compared to those obtained with methaqualone, glutethimide, and/or phenobarbital.

All substances (suspended in 0.5% carboxymethyl cellulose soln) were submitted to a preliminary screen in mice to determine effects on behavior.[7,8] Initial studies on lethality of a few selected compds (following ip administration to mice) indicated that a general

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(2) K. Winterfeld and H. Schueler, *Arch. Pharm.*, **293**, 203 (1960).

(3) J. R. Norton, A. A. Benson, R. A. Seibert, and F. W. Bergstroem, *J. Amer. Chem. Soc.*, **68**, 1330 (1946).

(4) H. Behringer and H. Meier, *Justus Liebigs Ann. Chem.*, **607**, 67 (1957).

(5) M. Freifelder and G. R. Stone, *J. Org. Chem.*, **26**, 3805 (1961).

(6) This material is probably formed by reaction of liberated methylmercaptan with unreacted **5**.

(7) S. Irwin, "Animal and Clinical Pharmacologic Techniques in Drug Evaluation," J. H. Nodine and P. E. Siegler, Ed., Year Book Publishers, Chicago, Ill., 1964, pp 36–54.

(8) G. Chen, *Symp. Sedative Hypn. Drugs*, **1953**, 45 (1954).