TABLE III
THE ESTIMATED METABOLIC PRODUCTS OF IMA IN DOG URINE

Compound	Dog 1 Concn, μg/ml	Dog 1 % of dose recovered	Dog 2 Concn, μg/ml	Dog 2 % of dose recovered
Methoxamine	30	8	25	5
2-Hydroxy-IMA	20	5	15	3
5-Hydroxy-IMA	100	26	110	22
2-Hydroxy-methoxamine	8	2	14	3

The mobility of this metabolite and 2-hydroxymethoxamine in system **1** was similar. The presence of this metabolite in the urine of IMA-treated dogs was confirmed by chromatographic means with **1**, 4, and 10; the mobility of the unknown and reference compound was the same in all systems. The identity of this metabolite could not be definitely established because the 5-O-demethylated methoxamine was not available and could not be compared with the possible metabolite, but the chromatographic evidence definitely showed the presence of an O-demethylated methoxamine derivative in the urine. The results of the estimation, in urine, of the metabolites of IMA are shown in Table III.

Methoxamine Metabolism in Dogs.—Methoxamine and the O-dealkylated metabolite of methoxamine were detected and estimated in urine samples from dogs treated with methoxamine by the technique described above for IMA-treated animals. The estimation of methoxamine and the O-demethylated methoxamine in the urine of two dogs treated with methoxamine is shown in Table IV.

Studies on the Possible N-Dealkylation of N-*t*-Butylmethoxamine.—The possible dealkylation of TMA to methoxamine was investigated. The urine from two TMA-treated dogs was analyzed for the presence of methoxamine. Methoxamine in the urine of these animals could not be detected by chromatographic means even though the method was sensitive to less than 0.2% of the dose. These results for the *t*-butyl derivative of methoxamine are in contrast to the results obtained for the isopropyl derivative.

TABLE IV
THE ESTIMATED METABOLIC PRODUCTS OF METHOXAMINE IN DOG URINE

Compound	Dog 1 Concn, μg/ml	Dog 1 % of dose recovered	Dog 2 Concn, μg/ml	Dog 2 % of dose recovered
Methoxamine	30	38	50	32
2-Hydroxy-methoxamine	10	12	15	10

Whether the presence of a hydrogen on the α carbon atom of the substituted alkyl group is a requirement for N-dealkylation was not investigated further. However, a wide variety of compounds have been demonstrated to undergo N-dealkylation in the intact animal as well as *in vitro*. For an excellent review of this subject, see the recent report of McMahon.[14]

Pharmacological Effects of Metabolites.—Methoxamine and IMA were rapidly converted to O-dealkylated metabolites in the animals studied. The 2-O-demethylated and 5-O-demethylated IMA both have activity in blocking the epinephrine-induced lipolysis in the rat epididymal fat pad.[1b] However, the compounds are not active *in vivo* presumably because they are rapidly conjugated and excreted.

N-isopropylmethoxamine undergoes cleavage of the N-alkyl group. This reaction is of considerable interest, since it can furnish an explanation of some of the side effects exhibited by animals that have been dosed with IMA. Animals that have received the N-*t*-butyl derivative of methoxamine, which is not dealkylated to methoxamine, do not exhibit these side effects.[15] Therefore, substitution of a *t*-butyl group for the isopropyl group blocked N-dealkylation as a metabolic pathway in this compound and eliminated the toxicity attributed to methoxamine.

Acknowledgment.—The authors gratefully acknowledge the aid, advice, and encouragement of Dr. John J. Burns of Hoffman-La Roche, Inc., Nutley, N. J., during the development of this work.

(14) R. E. McMahon, *J. Pharm. Sci.*, **55**, 457 (1966).
(15) J. J. Burns, S. A. April, and R. A. Salvador, *Progr. Biochem. Pharmacol.*, **3**, 248 (1966).

Local Anesthetics with Enhanced Affinity for Proteins

J. HOLLOWOOD, A. B. A. JANSEN, AND P. J. SOUTHGATE

John Wyeth & Brother Ltd., Institute of Medical Research, Taplow, Buckinghamshire, England

Received March 20, 1967

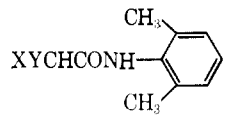
2-Dialkylaminoaceto-2′,6′-xylides bearing groups with some affinity for protein, such as CH_2SH, $CH_2CH{=}CH_2$, $CH_2C{\equiv}CH$, CH_2OH, $COOC_2H_5$, CH_2OOCCH_3, and $CONH_2$, in the 2 position have been synthesized and evaluated as local anesthetics.

Many local anesthetics, *e.g.*, lidocaine (**1**), have in common the molecular feature, $R_2N\cdots CO$, in which the basic nitrogen atom is separated from the carbonyl group by a chain of 1–4 atoms,[1] but we are aware of no specific attempt to increase the affinity of the molecule for tissue protein by attaching thereto potential binding groups such as SH (to form a disulfide bond), $CH{=}CH_2$ or $C{\equiv}CH$ (sulfide bond), OH (hydrogen bond), or dipoles like $COOC_2H_5$, $OOCCH_3$, and $CONH_2$ (van der Waals forces). Such groups, we conceived, by helping to retain the drug at the injection site, might both prolong its duration of action and reduce side effects on the central nervous system. We report here upon the

(1) A. Burger in "Medicinal Chemistry," A. Burger, Ed., 2nd ed, Interscience Publishers, Inc., New York, N. Y., 1960, p 441.

synthesis and evaluation of some compounds of this pattern.

Chemistry.—For the greater part, our synthetic route has involved the preparation of the appropriate N,N-dialkylamino acids and their coupling with 2,6-xylidine. Acids of this type, unlike amino acids, have been relatively little studied and general synthetic methods are lacking. Thus, only one compound of the type we required, *viz.*, N,N-dimethylcysteine, an intermediate for the lidocaine analog (**2**), has been described.[2] The reductive methylation, we found, proceeded better with the S-benzyl derivative, which in any event we required for the next stage, than with



	X	Y		X	Y
1,	H	$N(C_2H_5)_2$	**10**,	$COOC_2H_5$	H
2,	CH_2SH	$N(CH_3)_2$	**11**,	$COOC_2H_5$	Br
3,	$CH_2CH{=}CH_2$	$N(C_2H_5)_2$	**12**,	$COOC_2H_5$	$N(C_2H_5)_2$
4,	$CH_2C{\equiv}CH$	$N(C_2H_5)_2$	**13**,	CH_2OH	$N(C_2H_5)_2$
5,	CH_2SH	$N(C_2H_5)_2$	**14**,	CH_2OOCCH_3	$N(C_2H_5)_2$
6,	$CH_2SCH_2C_6H_5$	$N(CH_3)_2$	**15**,	$CONH_2$	H
7,	$CH_2SCH_2C_6H_5$	$N(C_2H_5)_2$	**16**,	$CONH_2$	Br
8,	$CH_2S{-}I_2$	$N(CH_3)_2$	**17**,	$CONH_2$	$N(C_2H_5)_2$
9,	$CH_2S{-}I_2$	$N(C_2H_5)_2$			

$(C_2H_5)_2NCX(COOR)_2$

	X	R
18,	NaC	C_2H_5
19,	$H_2CH{=}CH_2$	C_2H_5
20,	$CH_2C{\equiv}CH$	C_2H_5
21,	$CH_2C{\equiv}CH$	t-C_4H_9
22,	H	t-C_4H_9
23,	$CH_2SCH_2C_6H_5$	t-C_4H_9

$(C_2H_5)_2NCHXCOOH$

	X
24,	$CH_2CH{=}CH_2$
25,	$CH_2C{\equiv}CH$
26,	$CH_2SCH_2C_6H_5$

cysteine itself. The N,N-diethyl homolog, a better candidate for our purpose, could not be obtained by this method, however.

A solution to the problem of potentially wide application was our finding that the well-known acetamidomalonate synthesis of amino acids can be extended to dialkylaminomalonates under appropriate conditions. Thus, as the first step to an olefinic analog (**3**) of lidocaine, the sodio derivative of diethyl diethylaminomalonate (**18**) in dimethyl sulfoxide was treated with allyl bromide to give the ester (**19**) in good yield, and this, on alkaline hydrolysis which was accompanied by decarboxylation, provided the acid (**24**).

For the synthesis of the acetylenic analog (**4**) a variation was necessary. Alkaline hydrolysis of diethyl diethylamino(propargyl)malonate (**20**), similarly prepared from propargyl bromide, gave only a small yield of the acid (**25**); the major reaction involved loss of diethylamine, presumably from an enamine, $>C{=}C{-}N(C_2H_5)_2COO{-}$, formed by prototropy. To overcome the difficulty, we prepared the corresponding *t*-butyl ester (**21**). Bromination of di-*t*-butyl malonate in the presence of $CaCO_3$, or better, esterification of bromomalonic acid with isobutene, provided the required bromo ester which reacted smoothly with diethylamine to give **22**, from which **21** was obtained by treatment of its sodio derivative with propargyl bromide. On gently warming with dilute mineral acid this ester was hydrolyzed and decarboxylated in good yield to **25**.

The di-*t*-butyl ester was also utilized for the synthesis of the N,N-diethylcysteine analog (**5**) of lidocaine. Treatment of its sodio derivative with benzylthiomethyl bromide (from the corresponding chloride with HBr) gave **23** which was smoothly hydrolyzed and decarboxylated with dilute acid to give **26** without any β elimination of benzyl mercaptan occurring.

Coupling of α-dialkylamino acids with NH_2 groups, predictably, does not follow closely the behavior of the stronger α-acylamino acids. For example, dicyclohexylcarbodiimide, one of the most generally applicable methods for peptide synthesis, completely failed to couple our acids with 2,6-xylidine. The ethoxyformic anhydride method,[3] however, though the yields were greatly inferior to those obtainable with α-acylamino acids, nevertheless served to produce the desired xylidides in quantity adequate for our purpose. The isolation of the products was greatly facilitated by the circumstance that their hydrochlorides were readily extractable from aqueous solution by $CHCl_3$.

The S-benzylxylidides (**6** and **7**) were readily debenzylated with sodium in liquid ammonia to provide the sulfhydryl compounds (**2** and **5**) and the corresponding disulfides (**8** and **9**).

Preliminary experiments toward the synthesis of the serine analog (**13**) of lidocaine by the malonic ester method were discouraging. Products, which were not isolated pure, from the interaction of methoxymethyl bromide and acetoxymethyl bromide with the sodiomalonic ester yielded much pyruvic acid on even mild acid hydrolysis. This decomposition, which seemingly involves decarboxylation, elimination of the β-oxy substituent, and finally hydrolysis of the resulting enamine, $CH_2{=}CN(C_2H_5)_2COOH$, is in marked contrast to what happens with a β-benzylthio substituent. The need to overcome these obstacles, however, disappeared because we had developed concurrently the following simple route to **13**.

Ethyl hydrogen malonate was coupled with 2,6-xylidine, using dicyclohexylcarbodiimide as the condensing agent, to give an excellent yield of 2-ethoxycarbonylaceto-2′,6′-xylidide (**10**). Bromination of **10** in $CHCl_3$ furnished the required 2-bromo-2-ethoxycarbonylaceto-2′,6′-xylidide (**11**); no 4′-bromoxylidide was detected. Treatment of the bromo compound with diethylamine in boiling ethanol yielded 2-diethylamino-2-ethoxycarbonylaceto-2′,6′-xylidide (**12**), reduction of which with $LiBH_4$ gave the desired hydroxyxylidide (**13**).

Other compounds which were prepared in this series were the acetoxyxylidide (**14**), prepared from **13** by acetylation and the carbamoylxylidide (**17**) which was prepared from **10** by treatment with ammonia to give the amide (**15**). Bromination gave the bromoamide (**16**) which on treatment with diethylamine gave the desired product (**17**).

Experimental Section[4]

L-S-Benzyl-N,N-dimethylcysteine.—A suspension of L-S-benzylcysteine (20 g) in water (1 l.) and formaldehyde (30 ml, 40%) was shaken with H_2 at room temperature and pressure in the presence of 10% Pd–C catalyst (10 g). Gas uptake ceased at approximately the theoretical volume. The filtered solution was evaporated under reduced pressure and the residue was digested with water (200 ml). After removal of the insoluble

(2) R. E. Bowman and H. H. Stroud, *J. Chem. Soc.*, 1342 (1950).

(3) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

(4) Precautions other had by 60–80°.

material the solution was again evaporated to dryness and the resulting solid was crystallized from ethyl acetate to give the dimethylamino acid as prisms (13.4 g), mp 150°, $[\alpha]^{20}D$ +117.45 (*c* 1, H_2O).

Anal. Calcd for $C_{12}H_{17}NO_2S$: C, 60.2; H, 7.2; N, 5.85; S, 13.4. Found: C, 60.2; H, 7.1; N, 5.7; S, 13.3.

Di-*t*-butyl Bromomalonate. a.—Bromine (26 ml) was added dropwise to malonic acid (50 g) in ether (37 ml). When uptake of halogen was complete most of the ether was distilled and the residual oil was placed in a vacuum desiccator over NaOH until it set to a solid crystalline mass. To this material in dry ether (100 ml), cooled in a freezing mixture, was added concentrated H_2SO_4 (5 ml) and liquid isobutylene (*ca.* 120 ml) and the resulting solution was kept in a pressure bottle at room temperature for 6 hr. The mixture was poured onto NaOH (75 g) in water (250 ml) and ice (250 g) and the organic layer was separated. The ester (62 g) was distilled, bp 90–100° (2.2 mm), $n^{22}D$ 1.4388.

Anal. Calcd for $C_{11}H_{19}BrO_4$: C, 44.7; H, 6.45; Br, 27.2. Found: C, 45.7; H, 6.3; Br, 26.6.

b.—Bromine (13.5 g) was added dropwise to a stirred solution of di-*t*-butyl malonate (100 g) in $CHCl_3$ (1 l.) with suspended $CaCO_3$ (46.5 g). After 1 hr at room temperature the mixture was refluxed (1 hr) until the bromine color was discharged and then filtered, washed with $KHCO_3$ solution, dried ($MgSO_4$), and evaporated. The residual oil (45 g) was distilled, bp 120–125° (11 mm).

Di-*t*-butyl Diethylaminomalonate (22).—A mixture of the bromo ester (45 g), diethylamine (40 ml), and ethanol (150 ml) was refluxed for 2 hr and then concentrated *in vacuo*. Water was added and the product was collected in ether, then extracted into 2 *N* HCl and finally extracted back again into ether after basification of the aqueous extract. After removal of the solvent the residual oil (36 g) was distilled, bp 94–100° (1.7 mm), $n^{22}D$ 1.4308.

Anal. Calcd for $C_{15}H_{29}NO_4$: C, 62.7; H, 10.2; N, 4.9. Found: C, 62.7; H, 10.3; N, 4.9.

Benzylthiomethyl Bromide.—HBr (17 g) was bubbled through a cooled solution of benzylthiomethyl chloride (12 g) in dry ether (20 ml). Removal of the solvent *in vacuo* left an oil that was sufficiently pure for the next stage, but for analysis a portion was distilled, bp 75–81° (0.1 mm).

Anal. Calcd for C_8H_9BrS: C, 44.25; H, 4.2; S, 14.8. Found: C, 44.5; H, 4.2; S, 14.50.

Preparations with Sodio Diethyl and Di-*t*-butyl Diethylaminomalonate.—Diethyl diethylaminomalonate[5] (0.05 mole) was added dropwise to a suspension of ether-washed NaH (0.055 mole, 50% in oil) in ether (50 ml). After the evolution of H_2 had ceased the ether was distilled and replaced by dimethyl sulfoxide (DMSO) (30 ml). With the di-*t*-butyl ester the sodio derivative was prepared directly in the reaction solvent since frothing was less serious than with the diethyl ester. The appropriate halide (0.05 mole) was then added and after 12 hr the mixture was poured into water and the product was collected in ether.

Diethyl Allyldiethylaminomalonate (10).—The ester, an oil (64%), bp 80–90° (0.4 mm), $n^{23}D$ 1.4485, was obtained from allyl bromide and the appropriate sodio derivative.

Anal. Calcd for $C_{14}H_{25}NO_4$: C, 62.0; H, 9.3; N, 5.2. Found: C, 62.0; H, 9.0; N, 5.3.

Diethyl Diethylamino(propargyl)malonate (20).—Propargyl bromide similarly gave an oil (78%), bp 85–90° (0.4 mm), $n^{23}D$ 1.455.

Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.0; H, 8.6; N, 5.2. Found: C, 61.95; H, 8.5; N, 5.3.

Di-*t*-butyl Diethylamino(propargyl)malonate (21) (81%), bp 103–110° (0.8 mm), $n^{22}D$ 1.4502, was similarly obtained.

Anal. Calcd for $C_{18}H_{31}NO_4$: C, 66.4; H, 9.6; N, 4.3. Found: C, 66.4; H, 9.5; N, 4.3.

Di-*t*-butyl Benzylthiomethyldiethylaminomalonate (23).—The crude ester (45%), obtained from interaction of the sodio ester with benzylthiomethyl bromide in tetrahydrofuran (THF), could be distilled only with considerable loss but was sufficiently pure for the next stage. A specimen, distilled for analysis, had bp 145° (0.05 mm).

Anal. Calcd for $C_{22}H_{37}NO_4S$: N, 3.4; S, 7.6. Found: N, 3.4; S, 7.5.

Hydrolysis of Malonic Esters. a. 2-Diethylaminopent-4-enoic Acid (24).—A mixture of the diethyl ester (35 g), 2 *N* NaOH (131 ml), and sufficient ethanol to give a single phase was heated on a steam bath under reflux for 5 hr. The solution was concentrated *in vacuo*, water was added, and the whole was extracted with ether. The aqueous phase was acidified slowly with 2 *N* H_2SO_4 to *ca.* pH 4 and then evaporated to dryness *in vacuo*. Digestion of the solid with ethanol, evaporation of the solution, and trituration of the residue with acetone gave the crystalline acid (11 g), mp 135°. A specimen, recrystallized from THF, had mp 135–136°.

Anal. Calcd for $C_9H_{17}NO_2$: C, 63.1; H, 10.0; N, 8.2. Found: C, 63.1; H, 9.9; N, 8.5.

b. 2-Dialkylaminopent-4-ynoic Acid (25).—The di-*t*-butyl ester (8.0 g) was heated on a steam bath with 2 *N* HCl (40 ml) for 1 hr and, after cooling, the solution was treated with De-Acidite FF resin (HCO_3^- form) until neutral. The liquor was washed with ether and then evaporated to dryness *in vacuo* to yield the crystalline acid (3.2 g), recrystallization of which from acetone afforded prisms, mp 130–132°.

Anal. Calcd for $C_9H_{15}NO_2$: C, 63.9; H, 8.9; N, 8.3. Found: C, 63.8; H, 8.9; N, 8.2.

c. DL-S-Benzyl-N,N-diethylcysteine (26).—The di-*t*-butyl ester (1.2 g), similarly treated, left a solid (0.56 g), mp 144–146° after recrystallization from ethyl acetate.

Anal. Calcd for $C_{14}H_{21}NO_2S$: C, 62.9; H, 7.9; N, 5.2; S, 12.0. Found: C, 62.7; H, 8.0; N, 5.3; S, 11.95.

2,6-Xylidides by Ethoxyformic Anhydride Coupling.—Ethyl chloroformate (0.02 mole) was added to an ice-cooled solution of the N,N-dialkylamino acid (0.02 mole) and triethylamine (0.02 mole) in $CHCl_3$ (12 ml), followed after 15 min by 2,6-xylidine (0.025 mole). A sluggish evolution of CO_2 began almost at once. After 2 days at room temperature the mixture was washed with $NaHCO_3$ solution and the solvent was evaporated. The residue was taken up in 2 *N* HCl, and the solution was washed with ether and then extracted ($CHCl_3$) into which passed the hydrochloride of the product. This was isolated in the usual way either as such or as the free base. The following xylidides were so prepared.

2-Diethylamino-4-enoyl-2′,6′-xylidide (3).—The hydrochloride separated as prisms (32%), mp 204°, from THF. A specimen, recrystallized from ethanol, had mp 209–210° dec.

Anal. Calcd for $C_{17}H_{26}N_2O \cdot HCl$: C, 65.8; H, 8.7; N, 9.0. Found: C, 65.5; H, 8.75; N, 8.9.

2-Diethylaminopent-4-ynoyl-2′,6′-xylidide (4).—The hydrochloride (42%), mp 198–200°, crystallized similarly from THF. A specimen, recrystallized from ethanol–ethyl acetate, had mp 202–204°.

Anal. Calcd for $C_{17}H_{24}N_2O_4 \cdot HCl$: C, 66.1; H, 8.2; N, 9.1. Found: C, 66.1; H, 7.9; N, 8.9.

L-S-Benzyl-N,N-dimethylcysteinyl-2,6-xylidide (6).—In this instance DMF (30 ml), rather than $CHCl_3$, was used as the solvent; obvious modifications were made for work-up. The free base, liberated from a gummy hydrochloride, slowly crystallized. Most of the contaminating oil was removed on a filter and the solid (25%), mp 80°, was washed with isopropyl ether. A specimen, recrystallized from 2-propanol, had mp 80–81°, $[\alpha]^{20}D$ +50.1° (*c* 1.95, EtOH).

Anal. Calcd for $C_{20}H_{26}N_2OS$: C, 70.15; H, 7.65; N, 8.2. Found: C, 70.0; H, 7.6; N, 8.1.

DL-S-Benzyl-N,N-diethylcysteinyl-2,6-xylidide (7).—The free base (31%), mp 81–86°, was obtained crystalline as above. A specimen, recrystallized from petroleum ether, had mp 88–90°.

Anal. Calcd for $C_{22}H_{30}N_2OS$: C, 71.3; H, 8.15; N, 7.6; S, 8.65. Found: C, 71.4; H, 8.1; N, 7.8; S, 8.8.

L-N,N-Dimethylcystinyl-2,6-xylidide (8).—Sodium (*ca.* 0.7 g) was added in small pieces to a suspension of the S-benzylxylidide (1.7 g) in liquid NH_3 (100 ml) until the blue color persisted. Sufficient NH_4Cl to discharge the color was added and the NH_3 was allowed to evaporate. Air was bubbled through the residue dissolved in water until the solution ceased to give a color with sodium nitroprusside. The solution was acidified with 2 *N* HCl and the product (as the hydrochloride) was extracted ($CHCl_3$). Evaporation of the solvent left the crystalline product (0.75 g), mp 213–216°, $[\alpha]^{20}D$ +122° (*c* 1, H_2O), after recrystallization from ethyl acetate.

Anal. Calcd for $C_{26}H_{38}N_4O_2S_2 \cdot 2HCl$: C, 54.2; H, 7.1; N, 9.7; S, 10.7. Found: C, 54.1; H, 7.4; N, 9.35; S, 10.3.

DL-N,N-Diethylcystinyl-2,6-xylidide (9).—The S-benzylxylidide (1.0 g) was treated as above to give the hydrochloride (0.8 g), mp 210° dec after recrystallization from 2-propanol.

15) *Cf.* E. R. H. Jones and W. Wilson, *J. Chem. Soc.*, 547 (1949).

Anal. Calcd for $C_{30}H_{46}N_4O_2S_2 \cdot 2HCl$: C, 57.0; H, 7.7; N, 8.9. Found: C, 56.7; H, 7.7; N, 8.7.

L-N,N-Dimethylcysteinyl-2,6-xylidide (2).—Sodium was added as before to the S-benzylxylidide (1.1 g) in liquid NH_3 (50 ml) and after discharge of the color with NH_4Cl, the NH_3 was evaporated in a stream of N_2. The residue was dissolved in dilute HCl from which O_2 had been boiled out and the solution was washed with $CHCl_3$ (to remove corresponding cystine derivative) and evaporated *in vacuo*. The residue was extracted with ethanol and the liquor, after removal of insoluble matter by filtration, was again evaporated to dryness. Repetition of the operation with acetone gave the cysteinylxylidide as the hydrochloride (0.67 g). A portion, recrystallized from 2-propanol, gave hygroscopic prisms, mp *ca.* 190° dec, $[\alpha]^{20}D$ +27.8° (*c* 1, H_2O).

Anal. Calcd for $C_{13}H_{20}N_2OS \cdot HCl$: C, 54.0; H, 7.3; N, 9.7; S, 11.05. Found: C, 54.3; H, 7.1; N, 9.3; S, 10.85.

DL-N,N-Diethylcysteinyl-2,6-xylidide (5).—The S-benzylxylidide (1.0 g) was debenzylated as above to give the hydrochloride (0.64 g), mp 189–192°, from 2-propanol.

Anal. Calcd for $C_{15}H_{24}N_2OS \cdot HCl$: C, 56.85; H, 7.95; N, 8.8; S, 10.1. Found: C, 56.15; H, 7.8; N, 8.4; S, 9.8.

2-Ethoxycarbonylaceto-2′,6′-xylidide (10).—Dicyclohexylcarbodiimide (76 g) was added to ethyl hydrogen malonate[6] (44 g) and 2,6-xylidine (41.5 ml) in CH_2Cl_2 (500 ml) at 0°. After 1 hr at 0° and 16 hr at room temperature the stirred mixture was treated with glacial acetic acid (5 ml) to decompose the excess carbodiimide. One hour later the dicyclohexylurea was filtered off and the solvent was removed *in vacuo* to give a solid (76 g) which was recrystallized from petroleum ether–ethyl acetate to yield the xylidide as colorless needles (70.1 g), mp 100–101°.

Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.4; H, 7.3; N, 6.0. Found: C, 66.3; H, 7.3; N, 6.1.

2-Bromo-2-ethoxycarbonylaceto-2′,6′-xylidide (11).—Bromine (3.5 ml) was added over a period of 0.5 hr to a well-stirred solution of the preceding xylidide (11.7 g) in $CHCl_3$ (100 ml) at room temperature. The mixture was stirred for a further 0.5 hr and then heated under reflux for 0.5 hr. The pale yellow solution was washed with $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated *in vacuo* to give a solid (15.1 g), recrystallization of which from ether furnished needles (11.8 g), mp 134–135°.

Anal. Calcd for $C_{13}H_{16}BrNO_3$: C, 49.7; H, 5.1; Br, 25.45; N, 4.5. Found: C, 49.6; H, 4.9; Br, 25.3; N, 4.4.

2-Ethoxycarbonyl-2-diethylaminoaceto-2′,6′-xylidide (12).—A mixture of the bromoxylidide (9.4 g) and diethylamine (6.6 g) in absolute ethanol (75 ml) was heated under reflux for 6 hr, then the ethanol, together with the excess diethylamine, was removed *in vacuo*. Water (100 ml) was added to the residue and the product was collected in ether. The crude base (8.4 g) remaining after evaporation of the solvent gave a sticky solid on treatment with ethereal HCl which crystallized from a 2-propanol–ether mixture in needles (7.1 g), mp 169–170°, after recrystallization from the same solvent pair.

Anal. Calcd for $C_{17}H_{26}N_2O_3 \cdot HCl$: C, 59.7; H, 8.0; Cl, 10.4; N, 8.2. Found: C, 59.3; H, 7.85; Cl, 10.0; N, 8.3.

Treatment of the above hydrochloride (3.4 g) with 2 *N* NaOH and recrystallization of the resulting free base from petroleum ether gave needles (2.75 g), mp 73–75°.

Anal. Calcd for $C_{17}H_{26}N_2O_3$: C, 66.7; H, 8.6; N, 9.2. Found: C, 66.6; H, 8.55; N, 9.1.

2-Diethylamino-2-hydroxymethylaceto-2′-6′-xylidide (13).—$LiBH_4$ (1.1 g) was added to the preceding ester (1.5 g) in dry THF (60 ml) and the mixture was heated under reflux for 48 hr. Water (10 ml) was added cautiously to decompose the excess borohydride, followed by 2 *N* NaOH (20 ml), and the mixture was extracted with ether. The dried ($MgSO_4$) extract was evaporated and the residual oil was treated with ethereal HCl. The resulting solid hydrochloride crystallized from ethyl methyl ketone in needles (1.1 g), mp 184–185°.

Anal. Calcd for $C_{15}H_{24}N_2O_2 \cdot HCl$: C, 60.05; H, 8.4; Cl, 11.8; N, 9.3. Found: C, 59.75; H, 8.5; Cl, 11.75; N, 9.2.

2-Carbamoylaceto-2′,6′-xylidide (15).—A suspension of 2-ethoxycarbonylaceto-2′,6′-xylidide (4.7 g) in 0.880 *N* NH_4OH (40 ml) was stirred at room temperature overnight. The resulting solid was filtered off, washed well with water, and recrystallized from ethanol–water to give needles (3.7 g), mp 183–184°.

Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.1; H, 6.85; N, 13.6. Found: C, 63.9; H, 6.8; N, 13.5.

2-Bromo-2-carbamoylaceto-2′,6′-xylidide (16).—Bromine (2.2 ml) was added gradually with stirring to 2-carbamoylaceto-2′,6′-xylidide (6.87 g) in $CHCl_3$ (50 ml) at room temperature and the mixture was then heated under reflux for 1 hr. The residue obtained after evaporation of the solvent *in vacuo*, crystallized from ether in needles (7.3 g), mp 196–197°.

Anal. Calcd for $C_{11}H_{13}BrN_2O_2$: C, 46.35; H, 4.6; Br, 28.0; N, 9.8. Found: C, 46.2; H, 4.5; Br, 28.2; N, 9.6.

2-Carbamoyl-2-diethylaminoaceto-2′,6′-xylidide (17).—The bromoxylidide (5.7 g), diethylamine (3.3 g), and absolute ethanol (40 ml) were heated under reflux for 6 hr and the solvent was then removed *in vacuo*. The residue was taken up in water (50 ml) and the product was extracted with three 50-ml portions of ether. Evaporation of the dried ethereal extracts left an oil (3.42 g) which on treatment with ethereal HCl, followed by crystallization of the resulting gummy solid from 2-propanol–ether, gave the hydrochloride as needles (2.1 g), mp 209–210°.

Anal. Calcd for $C_{15}H_{23}N_3O_2 \cdot HCl$: C, 57.6; H, 7.75; N, 13.4. Found: C, 57.8; H, 7.95; N, 13.2.

2-Acetoxymethyl-2-diethylaminoaceto-2′,6′-xylidide (14). 2-Hydroxymethyl-2-diethylaminoaceto-2′,6′-xylidide (2.64 g), Ac_2O (2 ml), and anhydrous pyridine (2 drops) were warmed on the steam bath for 15 min and then poured into water (100 ml). After *ca.* 16 hr the solid that separated was filtered, dried, and recrystallized from dilute AcOH to give needles (2.71 g), mp 98–100°.

Anal. Calcd for $C_{17}H_{26}N_2O_3$: C, 66.7; H, 8.6; N, 9.2. Found: C, 66.75; H, 8.65; N, 9.1.

The hydrochloride was prepared from the above acetate (2.7 g) in the usual manner and recrystallized from 2-propanol–ether to give needles (1.4 g), mp 202–203°.

Anal. Calcd for $C_{17}H_{26}N_2O_3 \cdot HCl$: C, 59.7; H, 8.0; N, 8.2. Found: C, 59.6; H, 8.3; N, 8.3.

Biological Methods.—Potency and duration of local anesthetic activity were assessed by the technique of Bülbring and Wajda.[7] Aliquots (0.1 ml) of 0.25, 0.5, and 0.1% solutions (pH 5–6) of the compounds in distilled water were injected intradermally into the backs of guinea pigs. A minimum of three animals was used for each compound. Local anesthesia was indicated by the absence of a flinching response when the treated site was pricked at 5, 15, 30, 60, 120, and 180 min after injection. Three days after the experiment the animals were killed and the treated areas were excised and examined histologically.

In a nerve block experiment in rats, 0.02 ml of 0.5 and 1.0% solutions were injected perineurally around the sciatic nerve. Anesthesia was assessed by the inability of the animals to splay the toes of the treated limb.

Tissue irritancy was determined by treating rats intravenously with Evan's blue, and assessing the inflammatory response to intradermal administration of 0.5 and 1.0% solutions of the compounds. Irritant compounds gave intense blue, edematous wheals. Lidocaine was used for comparison throughout the experiments.

Results

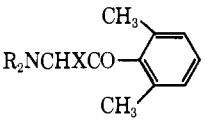
Table I lists the compounds which were tested for local anesthetic activity. Four of these (**3**, **4**, **8**, and **13**) had a prolonged local anesthetic action compared with lidocaine. With **4** and **8** where the duration of local anesthesia was in excess of 2.5 hr the injection site was inflamed and edematous. Subsequent histological examination of the areas showed acute inflammatory response with ulceration, proliferation of fibroblasts, and occasional fibrosis with underlying muscle involvement.

Compound **3** at concentrations lower than 1%, and **13** at 1%, gave anesthesia of longer duration than that produced by lidocaine but caused only minimal tissue damage. Because of this observation both compounds were examined in the nerve block and irritancy tests. Compound **3** induced a nerve block of 45–90 min, which was marginally larger than that produced by lidocaine

(6) R. E. Steele in "Organic Syntheses," Coll. Vol. IV, N. Rabjohn, Ed., John Wiley and Sons, Inc., New York, N. Y., 1963, pp 417–419.

(7) E. Bülbring and I. Wajda, *J. Pharmacol. Exptl. Therap.*, **85**, 78 (1945).

TABLE I
BIOLOGICAL RESULTS



No.	X	R	Potency ratio at 1 hr	Time (min) to 50% anesthesia at conen of 1%	0.5%	0.25%	Tissue damage
1	H (lidocaine)	C_2H_5	1	68	52	41	Slight
3	$CH_2CH{=}CH_2$	C_2H_5	1	>240	105	45	Severe at 1%
4	$CH_2C{\equiv}CH$	C_2H_5	3	205	180	160	Severe at all levels
2	CH_2SH	CH_3	0	27	0	0	Slight
8	CH_2S-I$_2$	CH_3	6	>240	>240	>240	Severe at all levels
5	CH_2SH	C_2H_5	0	0	0	0	Slight
9	CH_2S-I$_2$	C_2H_5	0	0	0	0	Slight
12	OOC_2H_5	C_2H_5	1	60	0	0	Slight
13	CH_2OH	C_2H_5	0.5	140	22	0	Slight
14	CH_2OOCH_3	C_2H_5	0.4	36	0	0	Slight
17	$CONH_2$	C_2H_5	0	0	0	0	Slight

(1%) (45–60 min). Compound **13** was active only at the 1% level and the effect was of shorter duration (30–60 min). In the irritancy test, 1% solutions of **13** and lidocaine gave no significant effects when compared with saline. Compound **3**, however, showed pronounced wheal formation at 1%, but was without effect at 0.5%.

In summary, therefore, of the ten compounds examined, four were without local anesthetic activity. Four compounds gave prolonged effects in the Bülbring and Wajda test; in two this was associated with irreversible tissue damage and in the nerve block test the duration of action of the other two did not differ markedly from that of lidocaine.

Acknowledgment.—We thank Mr. B. Basil for some of the pharmacological results and Mr. P. W. Tipton and Mrs. M. de Bruin for skillful technical assistance.

Hycanthone,[1] a New Active Metabolite of Lucanthone[2]

D. ROSI, G. PERUZZOTTI, E. W. DENNIS, D. A. BERBERIAN, H. FREELE, B. F. TULLAR, AND S. ARCHER

Sterling-Winthrop Research Institute, Rensselaer, New York

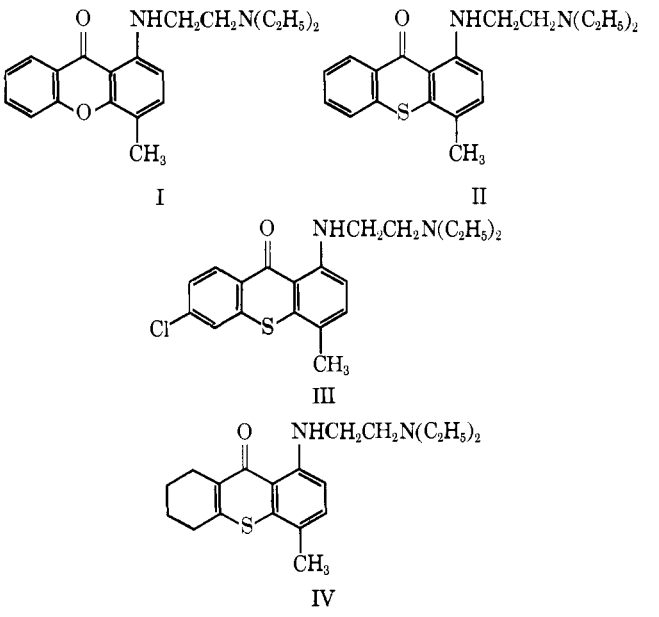
Received February 2, 1967

Microbiological oxidation of lucanthone[2] furnished hycanthone,[1] the 4-hydroxymethyl analog as the main product. Hycanthone is a highly active schistosomicidal agent when given orally or intraperitoneally. It has been identified chromatographically in the urines of man, monkey, and mouse following medication with lucanthone. Its chemical, physical, and biological properties suggest that it is the active metabolite of lucanthone.

Almost thirty years ago Mauss[3] synthesized a series of xanthenones, some of which were shown by Kikuth and Gönnert[4] to have schistosomicidal activity when administered orally to mice infected with a Liberian strain of *Schistosoma mansoni*. This was a signal chemotherapeutic achievement because this was the first orally effective nonmetallic organic compound found to possess such biological activity.

The most interesting members from the point of view of structure–activity relationships were miracil A (I), lucanthone (II), and the 6-chloro analog (III).

On the basis of evaluation in mice it was estabished that the thioxanthen-9-one II was more effective than



I II III IV

(1) For a preliminary communication see D. Rosi, G. Peruzzotti, E. W. Dennis, D. A. Berberian, H. Freele, and S. Archer, *Nature*, **208**, 1005 (1965). Hycanthone is the generic name for 1-[2-(diethylamino)ethylamino]-4-hydroxymethylthioxanthen-9-one.

(2) Lucanthone is the generic name for miracil D.

(3) H. Mauss, *Chem. Ber.*, **81**, 19 (1948). Although the chemistry was reported after World War II, it is well known that the original work was carried out in the late 1930's.

(4) W. Kikuth and R. Gönnert, *Ann. Trop. Med. Parasitol.*, **42**, 256 (1940).