Benzo[b]thiophene Derivatives. XVI. The Sulfur Isosteres of Melatonin, Bufotenine, 5-Hydroxytryptophan, and Related Structures[1a,b]

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The isosteric relationship which exists between indole and benzo[b]thiophene has created considerable interest in the syntheses and pharmacological properties of benzo[b]thiophene analogs of biologically active indole derivatives.[2-6] In connection with this interest we recently reported on the synthesis and pharmacology of the S analog of serotonin (SAS).[7,8] We now report on the syntheses of the benzo[b]thiophene analogs of 5-hydroxytryptophan (IV), melatonin (VId), bufotenine (VIIIa), and some closely related derivatives, with preliminary pharmacological results.

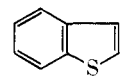
The key intermediate required for the preparation of IV was 5-benzoyloxy-3-bromomethylbenzo[b]thiophene (I) described in a previous paper.[8] Condensation of I with diethyl formamidomalonate gave the crude ester IIa in quantitative yield[9] according to a modified procedure of Avakian.[10] The triester was hydrolyzed and decarboxylated[11] to produce the desired amino acid isostere IV in 39% overall yield from I.

After converting I into 5-hydroxy-3-cyanomethylbenzo[b]thiophene (Va),[8] we encountered some difficulty in preparing Vb. Neither NaOH in Me_2SO_4 nor CH_2N_2 and $BF_3 \cdot Et_2O$ in Et_2O would afford methylation of the phenolic OH. This was overcome, however, by preparing the solid Na salt of Va and then refluxing it in DMF containing MeI. Vb was reduced to VIb, the methoxy S analog of serotonin, and acetylated to VId, the S analog of melatonin. In a similar fashion Va was converted into VIa,[8] and the 5-hydroxy melatonin derivative VIc.

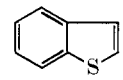
VIIIa, the S analog of bufotenine, was obtained from Va *via* the hydroxy acid chloride and dimethyl amide, VIIc. Similarly the methoxy S analog of bufotenine, VIIIb, was prepared from Vb. Attempts to demethylate VIIIb to VIIIa *via* either BBr_3 in CH_2Cl_2 at $-80°$[12] or with HBr and HOAc at reflux[13] failed, reminiscent of attempts to prepare VIa *via* debenzylation of 3-(β-aminoethyl)-5-benzyloxybenzo[b]thiophene.[14] The above reactions are summarized in Chart I.

The nmr data for the prepared compounds are presented in Table I. Although different solvents had to be employed because of solubility limitations, this should not affect our general interpretations discussed below.

The aromatic protons H_2, H_4, H_6, and H_7 obeyed first-order splitting rules and gave the anticipated J values.[15] However, as has been observed in other 5-substituted benzo[b]thiophenes[16,17], $\Delta(\delta H_6 - \delta H_4)$ was not zero as would be expected of the protons in an ortho-substituted benzenoid system. We could attribute this to two main factors: first, the different side chains on C_3 should have a *peri* interaction with H_4, and second, the C_3–C_5 bond order (0.71) is greater than the C_5–C_6 bond order (0.62)[18] making structure A a more important resonance contributor than B. Both of these factors would



A



B

favor a greater variance in H_4 over H_6 as was observed in the table.

The side-chain protons, Ha and Hb, fell into three categories. In IV, VIc, and VId the $\Delta\delta$ values were much greater than J so we could observe the splitting patterns. In IV, Hb appeared as a clean triplet but the Ha's were observed as a multiplet. Irradiation at Hb caused the Ha's to collapse to a broad singlet. However, when the same experiment was tried with 5-HTP (analytical material from Regis Chem. Co.) the Ha's collapsed to an AB quartet. This indicated that the Ha protons on 5-HTP were more nonequivalent than those on IV, although the latter were not equivalent since they appeared as a broad singlet upon irradiation and first-order splitting was not observed in the original spectrum. In VIc and VId we observed a triplet–quartet pattern for the Ha and Hb protons. Double resonance experiments confirmed that the Hb quartet was caused by coupling with the Ha protons and the amide proton.[19] A close look at the Ha triplet revealed

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(1) (a) Contribution No. 1810. For part XV of this series see E. Campaigne, A. Dinner and E. S. Neiss, *J. Heterocycl. Chem.*, **7**, 695 (1970); (b) This work was supported by Public Health Service Research Grant GM-10366 to Indiana University; (c) Taken in part from the thesis to be submitted by A. Dinner in partial fulfillment of the requirements for the PhD. degree at Indiana University.

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(9) When the condensation was tried with diethyl acetamidomalonate, IIb was formed in 50% yield. The ester could be hydrolyzed in base to III but this diacid could not be decarboxylated to IV.[2] Chapman has recently noted that α-acetamido-β-(3-benzo[b]thienyl)propionic acid could only be hydrolyzed to the α-amino acid by boiling it with 30% aqueous NaOH for 20 hr. (N. B. Chapman, R. M. Scrowston, and R. Westwood, *J. Chem. Soc.*, 1855 (1969).

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Chart I

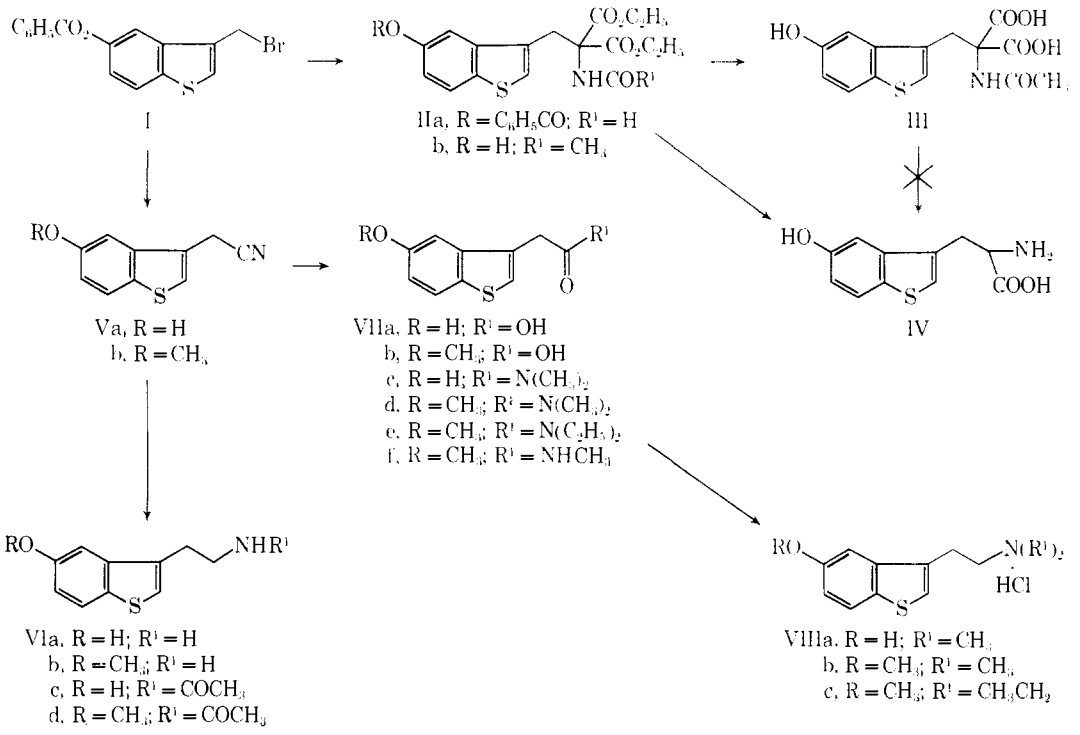
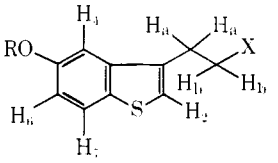


Table I

Nmr Data for 3,5-Disubstituted Benzo[*b*]thiophenes



	H_2[a]	H_4[a]	H_6[a]	H_7[a]	IIa[b]	IIb[b]	R	Solvent[c]
IV	7.58[d]	7.28	7.09	7.79	3.55 (m, 6)	4.54 (t, 6)		D
5-HTP[e]	7.26	7.10	6.83	7.34	3.38 (m)	4.20 (m)		D
Vb	7.39	7.04	7.00	7.68	3.75 (d, 2)[f]		3.85	A
VIa[g,h]	7.45	7.29	7.10	7.74	3.38 (m)	3.38 (m)		D
VIb	7.54	7.41	7.09	7.82	3.33 (m)	3.33 (m)	3.95	B
VIc	7.29	7.35	6.92	7.67	2.99 (t, 6.5)	3.42 (q, 6.5)		C
VId	7.12	7.22	7.00	7.68	3.02 (t, 6.5)	3.58 (q, 6.5)	3.88	A
VIIa[g]	7.46	7.27	7.00	7.70	3.84 (d, 2)[f]			C
VIIb	7.50	7.35	7.00	7.75	3.86 (s)		3.86	C
VIIc	7.35	7.13	6.86	7.66	3.80 (s)		9.36	B
VIId	7.19	7.27	6.98	7.66	3.84 (s)		3.87	A
VIIe	7.18	7.26	6.96	7.64	3.81 (d, 1)[f]		3.84	A
VIIf	7.29	7.14	7.00	7.70	3.77 (s)		3.85	A
VIIIa	7.41	7.31	7.02	7.68	3.54 (t, 8)[f]	3.55 (m)[f]		D
VIIIb	7.47	7.35	6.97	7.62	3.36 (t, 8)[f]	3.57 (t, 8)[f]	3.95	D
VIIIc	7.50	7.35	7.06	7.74	*j*	*j*	3.97	D

[a] The multiplicity and J values for these protons are the same for each compound and are as follows: H_2, slightly broad s; H_4, d, $J_{46} = 2 \pm 0.5$ Hz; H_6, d of d, $J_{64} = 2 \pm 0.5$, $J_{67} = 8 \pm 1$; H_7, d, $J_{76} = 8 \pm 1$. [b] The first value in parentheses after the δ value gives the multiplicity, the second gives the J value (Hz). [c] A, $CDCl_3$; B, DMSO-d_6; C, $(CD_3)_2CO$; D, D_2O-DCl. [d] Chemical shifts were determined on a Varian HA-100 spectrometer using TMS or TMSP (for solvent D) as internal lock. [e] Purchased from Regis Chem. Co., analytical purity. [f] This value is for J_{ab}. [g] This compound is known, but its nmr spectrum has not been reported. [h] 60 MHz nmr. [i] 220 MHz nmr. [j] This value was obscured by the CH_2 resonance of the Et groups.

that each peak was split into a fine doublet which double resonance showed was caused by allylic coupling to the H_2 proton. In fact, in all compounds prepared this allylic coupling was present.[20]

In VIa and VIb the Ha and Hb chemical shift differences were of the order of J so no information could be gathered concerning δ or J values.

(20) E. Campaigne and E. S. Neiss, *J. Heterocycl. Chem.*, **2**, 231 (1965).

In VIIIa and VIIIb the chemical shift difference relative to the J value was large enough so that with the aid of a 220-MHz instrument we could obtain J and δ values.

Biological Evaluation.—Twenty rats were pretreated with iproniazid (50 mg/kg base, po). Twenty-two hours later, 5 rats were given 5-HTP (55 mg/kg, ip), 1 rat received 110 mg/kg, ip. These rats showed marked tremors and piloerection within 15 min. The

sympathomimetic response progressed to clonic convulsions and death. This procedure was repeated using the S analog. There were no sympathomimetic effects evident at 30 min, at which time 2 rats were given 55 mg/kg of 5-HTP. There was no protection. One hr after the 110-mg/kg 5-HTP dose, the rat was challenged with 55 mg/kg of 5-HTP. There was no protection. The results, based on the fact that 5-HTP is converted *in vivo* into serotonin whose oxidation is then inhibited by an MAO inhibitor, indicate that the S analog neither mimicked nor blocked the effects of 5-HTP.[21] Since it has been shown previously that the lethal effects of VI, SAS, are greatly potentiated by pretreatment of mice with an MAO inhibitor[7] it seems probable that IV is not decarboxylated *in vivo*. Further CNS tests (anti-Tremorine and antinicotine in mice) with IV yielded negative results although 5-HTP was also inactive in these tests.[22]

Preliminary testing has been completed on VIIIa, VIIIb, VIIIc, and VIb. Three mice each were injected ip at different dose levels and their behavior was observed as presented in Table II.[23] While it took

Table II
Preliminary Biological Data for Some 5-Oxygenated 3-β-Aminoethylbenzo[b]thiophenes

Compd	LD_{50} (mice, ip, free base, mg/kg)	Dose level [mmoles/kg (free base)] 25	50	100	200	400
VIIIa	155	+[a]	d	dd	C, D(2/3)	C, D(3/3)
VIIIb	158	+	++	++, C	C, D(2/3)	C, D(3/3)
VIb	123	s	ss	ss, t	C, D(3/3)	C, D(3/3)
VIIIc		−	−	s	C, D(3/3)[b]	C, D(1/1)

[a] (+) Slight hyperactivity; (++) definite hyperactivity; (d) slight depression; (dd) definite depression; (s) slight sedation; (ss) definite sedation; (t) tremors; (C) convulsions; (D) death, parentheses give number in trial that died; (−) no apparent effect. [b] Dose level was 300 mmoles/kg (free base).

about 30 min at the 3 lowest dose levels for VIIIa to show activity and 15 min for the deaths to occur, the activity of VIIIb was apparent very fast at low dose levels and the deaths occurred within 1 min of injection. These findings are in accord with those of Gessner and Page[24] who showed that methoxybufotenine acted faster and more effectively than bufotenine in a CNS test designed to measure the conditioned avoidance response of trained rats. Screening of these indole isosteres is continuing.

The melatonin analog, VId, had no specific antifertility effect on the male rat in a preliminary screen.[25] The compound was implanted subcutaneously in male rats in a silastic tubule which permitted sustained release. Animals were castrated unilaterally at weekly intervals to determine effect on testicular function and histological damage to semeniferous tubules. Some loss of weight in the testes and signs of exfoliation were observed. After 8 weeks, the remaining animals were mated without difficulty, indicating no specific effect on Leydig cells nor specific interference with sperm physiology. All female mates delivered normal sized litters of healthy pups.

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(25) We are indebted to Dr. K. A. Laurence, Associate Director, Bio-Medical Division, The Population Council, Rockefeller University, N.Y., for the description and results of this test.

Experimental Section[26]

Ethyl 5-Benzoyloxy-3-benzo[b]thienylformamidomalonate (IIa).—To a solution of 0.45 g (19.6 mmoles) of Na in 100 ml of abs EtOH was added 3.75 g (18.5 mmoles) of diethyl formamidomalonate (Aldrich Chemical Co.) followed 5 min later by 6.45 g (18.5 mmoles) of I. The light yellow solution was allowed to reflux for 3 hr whereupon it was diluted with 500 ml of an ice–H_2O mixture and extracted with four 100-ml portions of Et_2O. The Et_2O was dried (Na_2SO_4) and evaporated to yield 8.65 g (100%) of a yellow oily solid which could be used directly to make the desired amino acid IV. An analytical sample prepared by recrystallization (abs EtOH) gave white needles, mp 140–141°. *Anal.* ($C_{24}H_{23}NO_7S$) C, H.

β-(5-Hydroxy-3-benzo[b]thienyl)-α-aminopropionic Acid (IV).—A suspension of 11.7 g (25 mmoles) of crude IIa in 75 ml of 10% NaOH was allowed to reflux for 5 hr after which time most of the formamidomalonate had dissolved. The hot yellow solution was filtered and 16 ml of concd HCl was added. A small amount of oil floated to the top of the solution and CO_2 was immediately given off. The mixture was allowed to reflux for another hour and was then decanted from the dark oil which stuck to the side of the refluxing flask. The solution, which now had a pH of 1–2, was cooled and the yellowish needle-like ppt that formed was collected and dried. Its ir(KBr) was identical with that of an authentic sample of BzOH (2.55 g, 85%). The light yellow filtrate was now made to pH 5–6 (NH_4OH) and concd to 0.5 its initial vol on a hot plate. Cooling afforded a white ppt, 4.1 g (39%). Recrystallization (hot H_2O) gave a white powder which darkened at 250° and melted at 268–270° dec. *Anal.* ($C_{11}H_{11}NO_3S$) C, H, N, S.

5-Methoxy-3-cyanomethylbenzo[b]thiophene (Vb).—Va (11 mmoles, 2.08 g) was dissolved in 120 ml of 0.1 *N* NaOH and the solution was evapd to obtain the solid Na salt of Va. This material was then dissolved in 20 ml of DMF containing 100 mmoles of MeI and refluxed for 3 hr. The resulting light yellow solution was diluted with 100 ml of a salt–H_2O–ice mixture and extracted with four 100-ml portions of Et_2O. The Et_2O was dried (Na_2SO_4) and evapd to give a yellow oil which solidified upon further pumping (0.3 mm, 4 hr). Recrystallization from EtOAc–petroleum ether (40–60°) gave white needles, 1.90 g (85%), mp 102–104°. The analytical material had mp 105–106°. *Anal.* ($C_{11}H_9NOS$) C, H.

5-Methoxy-3-(β-aminoethyl)benzo[b]thiophene·HCl (VIb).—To LAH (0.228 g, 6 mmoles) in 40 ml of dry Et_2O was added quickly 0.80 g (6 mmoles) of $AlCl_3$ in 65 ml of dry Et_2O. Five minutes after the last addition of $AlCl_3$, 2 mmoles of Vb in 65 ml of dry Et_2O was added slowly to the stirred mixture. Ten minutes after the last addition of nitrile the excess reducing agent was decompd carefully with H_2O and then the mixture was poured into 100 ml of HCl (pH 5). The Et_2O layer was sepd and the aq phase was extracted with fresh Et_2O at pH 6, 8, 10, and 14. The combined Et_2O fractions were dried (Na_2SO_4) and dry HCl gas was bubbled into the clear solution. Fine white needles pptd (0.29 g, 60%), mp 206–207° from MeOH–EtOAc. *Anal.* ($C_{11}H_{14}ClNOS$) C, H, Cl, S.

5-Methoxy-3-(β-acetaminoethyl)benzo[b]thiophene (VId).—A solution of 0.486 g (2 mmoles) of VIb in 7 ml of H_2O was shaken with 6 mmoles of Ac_2O, then 6 mmoles of NaOAc in 2 ml of H_2O was added. The mixture, from which an oil sepd, was then diluted with H_2O, extracted with Et_2O, dried ($MgSO_4$), and the Et_2O evapd. The residue was recrystd (ETOAc–petroleum ether) to give white needles, 400 mg, 80%, mp 99–100°. *Anal.* ($C_{13}H_{15}NO_2S$) C, H, N, S.

5-Hydroxy-3-(β-acetaminoethyl)benzo[b]thiophene (VIc).—This material was prepared similarly to VId. The fine white needles were recrystd from MeOH–CCl_4, yield 55%, mp 165–166°. *Anal.* ($C_{12}H_{13}NO_2S$) C, H, N, S.

(26) Melting points were measured on a Mel-Temp capillary melting point apparatus and are corrected. Ir spectra were determined with a Perkin-Elmer Model 137 Infracord and were as expected. The microanalyses were performed by Midwest Microlabs, Inc., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

5-Methoxy-3-benzo[*b*]thiopheneacetic Acid (VIIb).—To 225 ml of 20% H_2SO_4 (prepared by adding 39 ml of concd acid to 267 ml of H_2O) was added 0.926 g (4.55 mmoles) of Vb. The mixture was refluxed with stirring for 48 hr after which time all of the nitrile had gone into solution. The solution was cooled and extracted with Et_2O (fraction A). The Et_2O was washed (10% $NaHCO_3$) and the aq phase carefully acidified (HCl) and extracted (Et_2O–fraction B). The Et_2O fractions were dried separately (Na_2SO_4) and evapd. No material was found in fraction A. Fraction B gave 0.735 g (73%) of off-white crystals. Sublimation afforded analytical material, mp 150–153°. *Anal.* ($C_{11}H_{10}O_3S$) C, H, O.

***N,N*-Dimethyl-5-methoxy-3-benzo[*b*]thienylacetamide (VIId).** To 40 ml of dry C_6H_6 containing 0.835 g (3.76 mmoles) of VIIb (not completely in solution) was added 2 g (15.7 mmoles) of oxalyl chloride and the greenish-yellow mixture was swirled and warmed to 55°. Within 60 min all material had gone into solution. The acid chloride remained as an oil after all volatile material had been removed under reduced pressure. Upon addition of 10 ml of C_6H_6 and then excess Me_2NH an exothermic reaction occurred. The C_6H_6 was then removed under reduced pressure and the residue dissolved in Et_2O, washed (H_2O), and dried ($MgSO_4$). Evaporation gave 0.89 g (95%) of amide which was purified *via* distillation at 140° (0.03 mm). The analytical material had mp 83–85°. *Anal.* ($C_{13}H_{15}NO_2S$) C, H, S.

***N,N*-Dimethyl-5-hydroxy-3-benzo[*b*]thienylacetamide (VIIc).** This material was made similarly to VIId. The analytical material (88%) was recrystd from C_6H_6, mp 163.5–165°. *Anal.* ($C_{12}H_{13}NO_2S$) C, H, S.

***N,N*-Diethyl-5-methoxy-3-benzo[*b*]thienylacetamide (VIIe).** This material was made similarly to VIId. The analytical material (81%) was distilled (130° 0.03 mm) to give a light yellow oil. *Anal.* ($C_{14}C_{15}NO_2S$)C, H, S.

***N*-Methyl-5-methoxy-3-benzo[*b*]thienylacetamide (VIIf).** This material was made similarly to VIId. The analytical material (94%) was sublimed (125°, 0.03 mm) to give white crystals, mp 137.5–139°; mol wt ($C_{13}H_{15}NO_2S$) calcd, 235.0667; found (mass spec), 235.0646.

5-Hydroxy-3-(β-*N,N*-dimethylaminoethyl)benzo[*b*]thiophene·HCl (VIIIa). To 0.79 g (3.35 mmoles) of VIIc in 175 ml of THF was added 0.54 g (14 mmoles) of LAH and the mixture was allowed to stir at 25° for 3.5 hr and then at reflux for 0.5 hr. Excess LAH was carefully destroyed (H_2O) and then 100 ml of dil acid was added to pH 5. THF was removed under reduced pressure and the solution was extracted (Et_2O) at pH 5, 8, 10, 12, and 14. The extracts were combined, dried ($MgSO_4$), and then saturated with HCl gas. Evaporation gave 0.73 g (85%) of a light yellow glass which was purified *via* distillation (185°, 0.01 mm). The material remained as a glass after purification. *Anal.* ($C_{12}H_{16}ClNOS$)C, H, Cl, S. A picrate was made by dissolving VIIIa in MeOH, adding a few drops of NH_3 and then boiling it with a saturated picric acid solution. Cooling afforded orange needles which were recrystd from MeOH, mp 199–200°. *Anal.* ($C_{18}H_{18}N_4O_8S$) C, H, N, S.

5-Methoxy-3-(β-*N,N*-dimethylaminoethyl)benzo[*b*]thiophene·HCl (VIIIb). To 1.54 g (6.2 mmoles) of VIId in 180 ml of dry Et_2O was added 4.5 g (12 *M*) of LAH and the mixture was allowed to reflux with stirring for 20 hr. Excess LAH was destroyed carefully (H_2O) and then 100 ml of 0.6 *N* NaOH was added. The whole mixture was filtered *via* suction and the trapped white salts were copiously washed with Et_2O. The NaOH solution was extracted (Et_2O) and all the extracts were then pooled, dried ($MgSO_4$), and said with dry HCl. Initially the solution turned cloudy but as the HCl addition continued it became clear. Evaporation afforded 1.04 g (60%) of product. Sublimation (160°, 0.01 mm) gave 0.95 g of analytical material, mp 174–175°, white crystals. *Anal.* ($C_{14}H_{18}ClNOS$) C, H, S.

5-Methoxy-3-(β-*N,N*-diethylaminoethyl)benzo[*b*]thiophene·HCl (VIIIc). This material was made similarly to VIIIa except Et_2O was used as the solvent. Sublimation (160°, 0.01 mm) of the crude product gave 0.55 g (85%) of white crystals, mp 187–189°. *Anal.* ($C_{16}H_{22}ClNOS$) C, H, Cl, S.

Anti-Tremorine Test. The compounds were administered as suspensions in gum tragacanth at 100 mg/kg sc to groups of 5 male Evans albino mice. Thirty minutes later Tremorine tartrate (30 mg/kg) was given ip. After a further 20 min the mice were placed individually in the tremor recording chamber which is a circular "Perspex" box (11.25 cm diameter × 11.25 cm high), mounted on a thin strip of foam rubber on a metal stand. The box was divided diametrically so that the mice were restricted to one-half of the box. A Devices dynamometer type UFI was positioned underneath the edge of the box so that the vertical sensing lever was applied with a force of 30*g*. The dynamometer output was recorded *via* a DC2C preamplifier (range 2–5 mV) on a Devices recorder. Tremor was recorded for a period of 30 sec and anti-Tremorine activity expressed as a per cent of the tremor activity of a group of mice treated with Tremorine only.

Antinicotine Test. This test was also carried out in groups of 5 male Evans mice. The test compound was administered sc suspended in gum tragacanth, and the control groups received equiv vols of saline. Thirty minutes later the mice were given a further injection of 1 mg/kg of nicotine base iv (as a solution in saline of the bitartrate). The number of mice showing clear clonic convulsions within 1 min of injection were recorded.

Isothiazolecarboxaldoximes and Methylated Derivatives as Therapeutic Agents in Poisoning by Organophosphorus Compounds

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The reaction of Me tosylate with (*E*)-isothiazole-3-carboxaldoxime (**1a**, pK_a = 9.8) and with (*Z*)-isothiazole-5-carboxaldoxime (**1c**, pK_a = 8.6) led to the corresponding hydroxyiminomethyl-2-methylisothiazolium tosylates **2a** (pK_a = 7.6) and **2c** (pK_a = 2.6), respectively. Oxidation of 4-methylisothiazole with CrO_3 produced isothiazole-4-carboxylic acid, which was converted into isothiazole-4-carboxaldehyde by a Reissert reaction. The *E* and *Z* isomers of isothiazole-4-carboxaldoxime (**1b**, pK_a = 10.3 and 10.9, respectively) failed to give a 4-hydroxyiminomethyl-2-methylisothiazolium salt with a variety of methylating agents. In combination with atropine sulfate, the oximes (*Z*)-**1c** and **2a** are therapeutically active against poisoning with Sarin in mice, whereas **2a** is also active against Paraoxon. Both (*Z*)-**1c** and **2a** are less toxic than 2-hydroxyiminomethyl-1-methylpyridinium methanesulfonate (P_2S) in mice.

Oximes are potent reactivators of esteratic enzymes, inhibited by organophosphorus compounds.[1] Among these oximes, 2-hydroxyiminomethyl-1-methylpyridinium methanesulfonate (P_2S) and 1,1′-trimethylenebis(4-hydroxyiminomethylpyridinium) dibromide (TMB_1) were found to be particularly effective, both *in vitro* and *in vivo*.[1,2] The isosterism of the pyridine and isothiazole ring systems[3] suggested a study of the antidotal activity of hydroxyiminomethyl-2-methylisothiazolium salts. The preparation of these compounds from iso-

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