Central Depressant Properties of 3,1-Benzoxazine Derivatives

Following our investigation of the pharmacological properties of some 1,3-benzoxazines[1], we extended our studies to a related family of 3,1-benzoxazines. This paper offers a preliminary account of the pharmacological activity of 13 members of the new series, as reported in the Table[2].

Compounds I, II, III were obtained by condensation of *o*-amino-benzyl alcohol[6], *o*-aminophenyldimethylcarbinol[7] and 2-(2-amino-3-methoxyphenyl) propan-2-ol[8] with $COCl_2$ in pyridine. Halogenation of I in AcOH with 1 or 2 moles of chlorine *viz* bromine afforded compounds IV, V, VI and VII[9], whereas nitration of I in conc. H_2SO_4 yielded VIII. Alkylation of I in $MeCO/K_2CO_3$ with CH_3I or isobutyl bromide for 120 h at 55 °C afforded IX and 4H-1-isobutyl-4,4-dimethyl-3,1-benzoxazine-2(1H)-one (oil) which on bromination gave X and XI. Compound XII was obtained by refluxing an EtOH solution of *o*-aminophenyldimethylcarbinol with CS_2 and a catalytic amount of KOH. Condensation of *o*-aminophenyldimethylcarbinol with NaCNO and dil. HCl gave 1-(*o*-(2-hydroxyisopropyl))-phenylurea (mp 169–170 °C) which on treatment with conc. HCl yielded compound XIII.

The compounds reported in the Table were first screened in male albino mice (19–21 g), studying the effect upon the CNS. Four mice were used per dose-level and the compounds were administered orally by gavage suspended in gum acacia (5%). The dose-levels were chosen on a logarithmic scale: 30, 100, 300 and 1000 mg/kg. The animals were dosed with 2 ml/100 g and observed 30, 90 and 300 min after drug administration.

After the general screening procedure we studied the antagonism of the listed compounds against chemically and electrically induced seizures. The agonist was administered at the moment of the maximal effect induced by the compounds. As agonist we used strychnine (0.75 mg/kg i.v.)[10], cardiazole (40 mg/kg i.v.)[11] and nicotine (3 mg/kg i.v.)[10]. The maximal electroshock (ELS) was induced by an adequate stimulator[12]. The original methods were slightly modified.

In the case of strychnine, the antagonism against death was used. As far as cardiazole, nicotine and ELS were concerned the inhibition of the tonic convulsions was considered as the parameter for the evaluation. The antagonists were given orally by gavage suspended in gum acacia (5%) at the volume of 2 ml/100 g. The ED_{50} were determined graphically[13].

The acute toxicity was determined using 10 mice per dose-level and administering the compounds orally by gavage suspended in gum acacia (5%) at a volume of 2 ml/100 g. The acute toxicity was concluded within 24 h. The LD_{50} was determined graphically[13].

The Table shows that compounds I–X induced depression of the CNS (general screening program) and a more or less severe hypothermia. Compound XI was inactive and compounds XII and XIII led to the appearance of tonic convulsions. The depression of the CNS was most pronounced in compounds IV and V, and to a lesser degree in compounds IX and X.

Chemically and electrically induced seizures were efficiently antagonized by compounds II–VII and IX and X.

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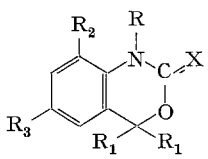

No.	X	R	R_1	R_2	R_3	mp (°C)	General screening: Depression of the CNS	General screening: Hypothermia	ED_{50} mg/kg orally: Strychnine 0.75 mg/kg i.v.	Cardiazole 40 mg/kg i.v.	Nicotine 3 mg/kg i.v.	Max ELS	LD_{50} mg/kg orally
I[3]	O	H	H	H	H	119–120	+++	++	200 i.a.	250	180	180	1200
II	O	H	CH_3	H	H	115–116	+++	+++	180	92	125	125	920
III	O	H	CH_3	OCH_3	H	96– 97	++	+++	380	490	260	470	>1000
IV	O	H	CH_3	H	Br	203–205	++++	++++	80	30	14	64	980
V	O	H	CH_3	H	Cl	200–202	++++	++++	175	35	33	90	1500
VI	O	H	CH_3	Br	Br	156–157	++	+	400	130	40	320	>1000
VII	O	H	CH_3	Cl	Cl	180–181	+	+	310	200	70	>200	1500
VIII	O	H	CH_3	NO_2	NO_2	175–176	+	+	>600	>600	240	>600	>2000
IX	O	CH_3	CH_3	H	H	95– 96	+++	+++	260	100	130	270	1500
X	O	CH_3	CH_3	H	Br	113–114	+++	++	160	80	43	130	2000
XI	O	i-butyl	CH_3	H	Br	78– 80	–	–	300 i.a.	100 i.a.	100 i.a.	–	>1000
XII	S	H	CH_3	H	H	134–135	–	–	100 i.a.	100 i.a.	100 i.a.	–	260
XIII[5]	NH	H	CH_3	H	H	144–145	–	–	i.a.	i.a.	i.a.	i.a.	240

It can be concluded that substitution in position 4 ($R_1 = CH_3$) increases the anti-convulsant activity of the parent compound whereas substitution in position 1 yields less active products. The activity is further enhanced by halogenation of the benzene nucleus.

All of the compounds (X–XI) showed a low acute toxicity on oral administration. An exception was presented by the last two compounds (XII and XIII).

The pharmacological and toxicological properties of these compounds seem to justify clinical trials. The pharmacological features of these compounds will be described extensively elsewhere.

Riassunto. Sono stati studiati 13 derivati della 3,1-benzossazina, la maggior parte dei quali sono risultati attivi come anticonvulsivanti. Tra di essi il più interessante è risultato il 4H-4-dimetil-6-bromo-3,1-benzossazine-2-one (IV).

L. Bernardi, S. Coda, A. Bonsignori, L. Pegrassi and G. K. Suchowsky

Department of Chemistry and Department of Pharmacology, Farmitalia, Istituto Ricerche, 20146 Milano (Italy), 25 March 1969.

An Azomethine-Hypophosphorous Acid Addition Compound as a new Colorimetric Reagent for Iron

For studies of tumour-inhibitory activity we prepared N,N′-*bis*-salicylidene derivatives of 1,3-diaminopropane and 1,2-diaminoethane[1] and the N-salicylidene compound of D-(+)-glucosamine[2]. Phosphonous acid derivatives were obtained from the azomethines by a general reaction: o-HOC_6H_4CH = NR + H_3PO_2 → o-$HOC_6H_4CH(PO_2H_2)$-NHR first described by Schmidt[3].

By chance it was found that aqueous solutions of the phosphonous acids gave stable red colours with ferric iron and the 1,2-diaminoethane compound proved to be most sensitive. Here we describe the preparation of this derivative and its use for the colorimetric determination of iron.

Materials, methods and results. N,N′-*bis*-salicylidene-1,2-diaminoethane phosphonous acid derivative [o-$HOC_6H_4CH(PO_2H_2)NH(CH_2)_2NHCH(PO_2H_2)C_6H_4OH$-o, SDEP].

N,N′-*bis*-salicylidene-1,2-diaminoethane (2.68 g; mp 127°) was dissolved in hot absolute ethanol (50 ml) and a slight excess of anhydrous hypophosphorous acid (~1.499; > 2 mole proportions) was added. Soon the yellow colour of the azomethine disappeared and a white precipitate formed. The mixture was refluxed for 10 min and kept overnight at room temperature. Precipitation of the phosphonous acid derivative was completed by addition of several volumes of anhydrous ether. SDEP was collected, washed with anhydrous ether and dried in air. Yield = 4 g; mp 206–207° with gas evolution.

Microanalysis showed that the compound contained water. A sample was dried at 60° in vacuo for 12 h and then analyzed. During weighing, the analytical sample gained several µg.

For $C_{16}H_{22}N_2P_2O_6 \cdot {}^1/_2H_2O$,

calcd. C% = 46.95; H% = 5.69; N% = 6.84
found C% = 46.77; H% = 5.63; N% = 7.17

Colour reaction of ferric iron with SDEP. Aliquots of a freshly prepared solution of ferric alum (analytical reagent grade: 0.4012 g in 100 ml of deionized water) were added to 5 ml portions of 0.4% (w/v) aqueous solution (pH ~3) of SDEP to give concentrations of ferric iron ranging from 0 to 15 µg/ml. Absorption spectra were determined against water by means of a Unicam SP 800 recording spectrophotometer. The coloured complex gave a prominent absorption maximum at 495 nm. At a concentration of 15 µg of Fe^{+++}/ml E was ~0.94. Beer's law was obeyed.

Over the pH range 3–8, E_{495} was practically constant. The colour was partially discharged when the pH was brought to 2 by hydrochloric acid but was restored when the pH was increased to 3 or over by addition of sodium hydroxide solution.

We examined the effect of various concentrations of SDEP (0.05–0.4 g/100 ml) on E_{495} of solutions containing 11 µg of Fe^{+++}/ml and found that E_{495} was not markedly influenced by SDEP concentration.

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Colorimetric determination of ferric iron directly and of ferrous iron (after hydrogen peroxide treatment) with SDEP reagent at 495 nm

Iron content (µg/ml)	I 0.4% SDEP + ferric	II 0.1% SDEP + ferric	III 0.1% SDEP + ferrous	IV 0.1% SDEP + ferrous + H_2O_2	V 0.1% SDEP + ferric + ferrous	VI 0.1% SDEP + ferric + ferrous + H_2O_2
	E ± S.D.	E ± S.D.	E	E ± S.D.	E	E
1.9	0.142 ± 0.100	0.127 ± 0.018	0.004	0.131 ± 0.005	0.075	0.158
3.9	0.259 ± 0.006	0.276 ± 0.010	0.004	0.281 ± 0.006	0.144	0.274
7.7	0.514 ± 0.012	0.541 ± 0.014	0.000	0.537 ± 0.011	0.283	0.545
11.4	0.736 ± 0.011	0.781 ± 0.013	0.002	0.786 ± 0.010	0.399	0.784
15.1	0.942 ± 0.015	1.034 ± 0.012	0.001	1.037 ± 0.005	0.520	1.042