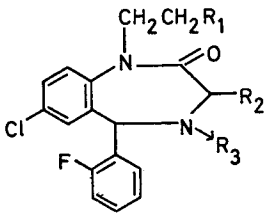
A new benzodiazepine: 1-(2-hydroxyethyl)-3-hydroxy-7-chloro-1,3-dihydro-5-(*o*-fluorophenyl)-2*H*-1,4-benzodiazepin-2-one

A series of new 1-hydroxyalkyl derivatives of 1,3-dihydro-2*H*-1,4-benzodiazepin-2-ones bearing a 3-hydroxyl or a 4-*N*-oxide function have been synthesized. One member, the title compound (III), was about 4 times less toxic and from 1·5 to 6 times more active than diazepam in a variety of tests in mice. We now report the preparation of this compound, together with some pharmacological results.



I R_1 = $OCOCH_3$; R_2 = H ; R_3 = O

II R_1 = $OCOCH_3$; R_2 = $OCOCH_3$; R_3 = -

III R_1 = OH ; R_2 = OH ; R_3 = -

Alkylation of 7-chloro-1,3-dihydro-5-(*o*-fluorophenyl)-2*H*-1,4-benzodiazepin-2-one-4-oxide (Earley, Fryer & others, 1968) with 2-bromoethyl acetate and sodium hydride in dimethylformamide gave I m.p. 161–163° (Found: C, 58·5; H, 4·3; N, 7·1; Cl, 9·2; F, 4·9: C_{19} H_{16} $ClFN_2O_4$ requires: C, 58·4; H, 4·1; N, 7·2; Cl, 9·1; F, 4·9%). By treatment of I with acetic anhydride the diester II was obtained, m.p. 129–130° (Found: C, 58·4; H, 4·1; N, 6·3; Cl, 8·1; F, 4·4: $C_{21}H_{18}$ $ClFN_2O_5$ requires: C, 58·3; H, 4·2; N, 6·5; Cl, 8·2; F, 4·4%). Ammonolysis of II, using methanolic ammonia, gave an 81% yield of the alcohol III (crystallizing from dichloromethane—light-petroleum (b.p. 40–60°), m.p. 138–140° (Found: C, 58·5; H, 4·0; N, 8·0; Cl, 10·3; F, 5·7: C_{17} H_{14} $ClFN_2O_3$ requires: C, 58·5; H, 4·1; N, 8·0; Cl, 10·2; F, 5·4%). The infrared and pmr spectra were consistent with the assigned structures.

When administered intraperitoneally to mice, the title compound (III) had an LD50 of 760 mg kg^{-1} compared with 185 mg kg^{-1} for diazepam. In tests in mice, the compound exerted depressant effects on spontaneous activity, irritability, limb and abdominal tone, as well as inducing increased positional passivity. In the electroshock seizure test (maximal), performed essentially by the method of Swinyard, Brown & Goodman (1952), compound III antagonized induced seizures; the ED50 was 3·0 mg kg^{-1} (i.p.) compared with that of diazepam (4·0 mg kg^{-1}, i.p.). The leptazol test was carried out as described by Everett & Richards (1944); leptazol was administered at a dose of 100 mg kg^{-1} (i.p.); the test compound, suspended in 5% gum arabic and administered 30–45 min before the induced seizure, showed an ED50 of 0·096 mg kg^{-1} (i.p.), compared with diazepam (0·63 mg kg^{-1} i.p.). A high antistrychnine activity was demonstrated after intraperitoneal administration 45 min before 3 mg kg^{-1} (i.p.) of the agonist; the ED50 was 9·8 mg kg^{-1} (diazepam 21·5 mg kg^{-1}).

In further experiments in mice, the test compound induced the loss of righting reflex when given intraperitoneally before a subhypnotic dose of hexobarbitone (20 mg kg^{-1}, i.p.); the ED50 was 0·60 mg $kg.^{-1}$ Diazepam was less potent in this respect, its ED50 being 1·45 mg kg^{-1}. The barbiturate sleeping time was significantly prolonged by

the compound in doses between 0·312 and 1·25 mg kg^{-1}. Variance analysis shows that at doses of 0·312 and 0·625 mg kg^{-1} it was significantly more active than diazepam ($P < 0·01$).

The experimental results indicate that the new compound exerts a central activity which appears to be superior to that of diazepam. Rats gave a pattern of responses similar to those seen in mice. Preliminary clinical assessment showed the drug to have activity in the treatment of minor and severe anxiety and associated symptoms.

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LETTERS TO THE EDITOR

Stability of anti-ricin serum

In view of the unsatisfactory nature of the microscopic test for castor seed, Clarke (1953) described a method of bioassay which made use of the fact that the serum from an immunised animal will neutralise the toxicity of ricin. The method is sensitive, and quite specific, but suffers from the disadvantage that few toxicological laboratories are likely to have the immune serum. It is not available commercially, needs special facilities to prepare, and is usually regarded as being comparatively unstable. There does not, however, appear to be any evidence for the latter supposition.

We have recently had occasion to test both the toxicity of a sample of ricin prepared in 1947, and the potency of some serum from a goat immunized at the same time. The former had been kept at room temperature, the latter preserved with 0·5% phenol and stored under refrigeration at 4°. Although the ricin had lost about 80 per cent of its original toxicity it still had an LD50 by intraperitoneal injection in mice of 2 mg kg^{-1}. 1 ml of the serum was still able to neutralize 8 mouse lethal doses of ricin and was thus quite potent enough to be used for the bioassay described. This means that the method is of much more practical value than was originally thought, as the serum, once obtained, can be stored in a refrigerator for many years without losing its essential efficacy.

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