STRUCTURE AND PSYCHOTROPIC ACTIVITY OF A NEW TETRAHYDROBENZODIAZEPINE CONGENER

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ABSTRACT

Assignment of the structure of a recently reported tetrahydro-1,5-benzodiazepine derivative is established by means of mass spectrometry (ms), proton magnetic resonance (pmr), examination of optical activity, and by chromatographic techniques. Analysis of the 100 MHz pmr spectrum revealed the presence of an ABC_3X spin system characteristic of the proton couplings of the cis form. Other analytical data are also consistent with this structure. Preliminary pharmacologic testing of this derivative indicated a considerable tranquilizing activity, which was evaluated via electroencephalographic (EEG) analysis in rabbits and potentiation of the effect of barbiturates in mice.

RESUME

La structure d'un nouveau dérivé de la tetra-hydro-1,5-benzodiazepine a été établie à l'aide des méthodes suivantes: spectrométrie de masse, résonnance magnétique protonique, activités optique et chromatographique.

Le spectre pmr pris à 100 MHz, révèle la présence d'un système de spin ABC_3X, caractéristique de couplages protoniques de la forme cis. D'autres mesures physiques ont confirmé cette structure. Des tests pharmacologiques préliminaires ont montré une activité tranquilisante considérable, évaluée par analyse électroencéphalographique chez le lapin et effet de potentiation des barbituriques chez la souris.

Increasing interest in the field of benzodiazepine chemistry is much influenced by the promising clinical utility of such psychotropic agents as diazepam[1], oxazepam[2] as well as other 1,4-benzodiazepines (1). Of the several factors affecting such activity, basicity of the benzodiazepine ring is being given much consideration (2). Indeed, certain tetra-1,4-benzodiazepine derivatives, with enhanced basic functions are recommended as efficient tranquilizing agents (3).

On the other hand, current interest in these laboratories in the complex metal-hydride reductions of five- and seven-membered heteroaromatic carbonium ions has led to the development of a new facile synthesis of pyrazolidines (4,5) and tetrahydro-1,5-benzodiazepines (6), through the sodium borohydride reduction of the corresponding pyrazolium and benzodiazepinium salts.

Apart from their inherent benzodiazepine skeleton, these recently reported saturated congeners (Structure I), might be endued a potential psychotropic activity, on account of their enhanced basic properties.

The present work is a preliminary report on the neuropharmacologic testing of 2,4-dimethyl-1H-2,3,4,5-tetrahydro-1,5-benzodiazepine (I, R – CH_3). As indicators of the potential tranquilizing activity, use is made of the potentiation of the hypnotic effect of pentothal sodium in mice, in addition to the changes brought about in the EEG patterns in rabbits. Assignment of the structural configuration of the investigated compound is also forwarded.

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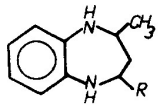
[1]Valium.
[2]Serax.



STRUCTURE I

CHEMISTRY

2,4-Dimethyl-1H-2,3,4,5,-tetrahydro-1,5-benzodiazepine, compound (I) was synthesised by reducing the corresponding benzodiazepinium salt with sodium borohydride in 95% ethanol according to the reported method (6). Structure of the reduction product was basically inferred from its microanalytical and infrared data, but without specifying the actual stereochemical configuration acquired after such a reaction. As reduction of the precursor benzodiazepinium cation can, in principle, lead to the formation of cis, trans and even racemic modifications of compound (I), it seems then reasonable to assign its actual configuration, especially before its pharmacologic investigation. In the present communication, assignment of the structure of compound (I) is established by means of thin layer chromatography, ms, pmr techniques and examination of optical activity.

PHARMACOLOGY

First, the toxicity of the investigated compound is determined in mice by estimation of its dose bringing about mortality in 50% of the animals, i.e. LD_{50}, that received various doses, administered intraperitoneally. Further, two methods are resorted to, for the tracing of the effect of the compound on the central nervous system (CNS): potentiation of the hypnotic effect of barbiturates in mice, and studying the effect of that particular compound on the EEG patterns in rabbits (7).

EXPERIMENTAL

Pmr spectra were determined in deuterochloroform on Varian A-60 A and 100 HA spectrometers, using tetramethylsilane as the internal reference. Ms data were supplied by an AEI MS 902 spectrometer, attached to an AEI DS 300 data system, and determined at 150° source temperature under 70 eV.

2,4-Dimethyl-1H-2,3,4,5-tetrahydro-1,5-benzodiazepine, (I)

The compound was prepared in 90% yield by reducing 2,4-dimethyl-1,5-benzodiazepinium chloride as reported. It was further purified by crystallization from petroleum ether, (40-60°) as colourless scales, mp 59-60°.

PHARMACOLOGIC EXAMINATIONS

1. Determination of the LD_{50}—In a group of 50 Alpino mice of both sexes, of an average weight of 20 g each, compound (I) was injected in the form of an aqueous sterile solution, the dose of which was calculated per kg animal body weight. Graphical representation of the percentage mortality observed after 24 hr versus the corresponding dose of compound (I) given afforded the LD_{50}.

2. Potentiation of the hypnotic effect of barbiturates—Four groups, each of 20 mice of the previous specifications, were used in this evalua-

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tion. In the first group, compound (I) was intraperitoneally injected in the form of four aqueous serial dilutions of 25, 50, 100 and 200 mg per kg animal body weight. In the second group, pentothal sodium[3], in a dose of 125 mg per kg body weight was similarly administered in combination with the above mentioned four doses of compound (I). The third group received only pentothal sodium, in the same dose as that of the second group, while the fourth group was left as a control. The general behaviour pattern was observed for the animals of the first group, while the sleeping time in minutes was recorded for animals of the second and third groups.

3. Effect on the EEG patterns—EEG measurements were carried out on a group of 10 Giza rabbits of either sex, of an average weight of 2 kg each, and using an 8-channeled Kaiser electroencephalograph. Three chronic nickel-chrome electrodes were implanted into the cerebral cortex through the appropriate holes in the skull bones (frontal, parietal and occipital areas). Bipolar electrodes were similarly introduced into subcortical levels (reticular formation, hippocampus, amygdala and red nucleus). The quantitative analysis of the EEG patterns was carried out according to Kaiser (8). Compound (I) was injected by rapid intravenous route as an aqueous solution of 5 mg per kg rabbit body weight. Registration of brain impulses lasted for 180 minutes.

RESULTS AND DISCUSSION

Chemistry

Apart from its apparent role in ascribing a selective feature for the borohydride synthesis of tetrahydrobenzodiazepines, the assignment of the actual configuration of the product(s) of this synthesis is quite essential, especially when a pharmacologic activity of this is being anticipated. Very often, configurational isomers show significant differences in their biologic effects.

The ms of the investigated compound, Fig. 1, revealed the existence of the molecular ion peak (M^+) with a relative abundance of 46.2% at m/e 176. This abundance is consistent with the structure of cyclic amines (9). The base peak, revealed at m/e 119, is evidently an ($M^+ - 57$) ion. As a 57-leaving fragment is mostly a $C_4H_9^{\cdot}$ or a successive contribution of $CH_3^{\cdot}$ and an olefin (10), and as fragmentation of the M^+ ion would not accommodate the formation of an intact $C_4H_9^{\cdot}$; a potential formation of an olefin should be then taken into consideration.

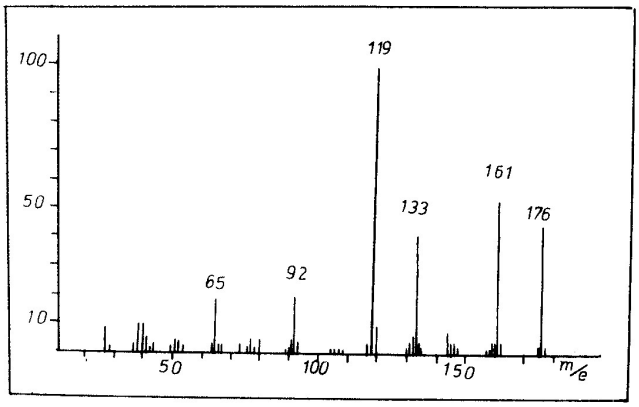
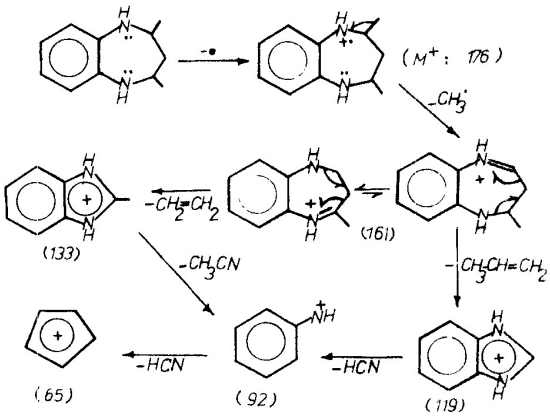


FIGURE 1—Mass spectrum of compound (I).

It is quite possible, that demethylation of the M^+ ion affords an $M^+ - 15$ ion, that suffers ring opening with the elimination of a neutral propene molecule. Such formation of the 119 m/e ion is substantiated by the presence of the corresponding metastable ion at 88.0 m/e, with a relative abundance of 2%, (m/e 161 ------→ m/e 119, m*, calculated: 87.95) and by the presence of the 161 m/e ion ($M^+ - 15$) in abundance more than 50%. As ring closure of the produced 119 diamine-ion seems to be an energetically favoured process, we suggest that the actual species of the 119 m/e ion is the benzimidazolium ion. The considerable thermodynamic stability of the latter cation was already reported (11). In addition, related ring opening with subsequent contraction into a benzimidazolium ion is characteristic of the mass spectra of triazepines (12). Fragmentation pathway of compound (I) can be thus illustrated by Scheme 1. The 133 m/e ion is suggested similarly to represent the 2-methylbenzimidazolium fragment; a metastable ion at 110 m/e substantiates such presence (m/e 161 ------→ m/e 133, m*, calculated: 109.86).



SCHEME 1—Fragmentation pathway of compound (I).

Further polarimetric investigation[4] of 4% (w/v) solution of compound (I) in 95% ethanol revealed no optical activity, thus suggesting the presence of either racemic and/or meso forms.

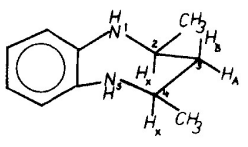
Thin layer chromatography[5] of the base or its in situ prepared (+)-tartaric acid salt, in the presence of such diverse solvent systems as ethanol-benzene(1:1), 1-butanol-acetic acid-water(4:1:5), and 2-propanol-formic acid-water(20:1:5) indicated the existence of a single, or an almost inseparable mixture of the cis and trans isomers.

The 100 MHz pmr spectrum of compound (I), Fig. 2, revealed a sharp singlet at τ 3.30 (4H of C_6H_4), another singlet at τ 6.72, that disappeared on addition of few drops of deuterium oxide, (2H of two equivalent N-H), an almost symmetrical eleven-line multiplet centered at τ 7.25, (2H of two C-H), a distorted multiplet centered at τ 8.45, (2H of nonequivalent CH_2), in addition to a doublet, (~6.5 cps) at τ 8.75 (6H of two equivalent CH_3).

The 2,4-protons of compound (I) can be regarded as the X portion of an ABC_3X spin system, the pmr analysis of which was already reported for some simpler systems, like the 2,6-dimethylpiperidines (13). Such analysis could help in the assignment of the cis or trans configurations in the following way: since $J_{AC} = J_{BC} = 0$, it is anticipated that the X resonance would consist of 4 lines (X part of ABX), each further splits into a 1:3:3:1-quartet by coupling with the methyl protons (C_3). Hence, the separation of the outer lines of the low-field multiplet (X-multiplet), Fig. 2, can be given by:

$$J_{ABC_3X} = J_{AX} + J_{BX} + 3J_{XC}$$

As this separation measured 32.5 cps, and as J_{XC} is observed to be 6.5 cps, it then follows that $J_{AX} + J_{BX}$ will be given as 13 cps. Further, J_{AX} is expected to measure ~3 cps- either of the cis or trans forms; since J_{AX} would equal either $\frac{1}{2}(J_{e_3a_2} + J_{e_3a_4})$ or $\frac{1}{2}(J_{e_3e_2} + J_{e_3a_4})$ respectively. This leaves J_{BX} to measure ~10 cps, which corresponds to the typical spin value of axial protons, and hence confirming the cis configuration; J_{BX} equals $\frac{1}{2}(J_{a_3a_2} + J_{a_3a_4})$, but is not equivalent to $\frac{1}{2}(J_{a_3e_2} + J_{a_3a_4})$.



STRUCTURE I—cis form.

[3]Nesdonal.

[4]Carl Ziess-20 polarimeter was used.

[5]Glass plates, (20 × 20 cm) with activated Kieselgel HF, Merck (0.75 mm), using iodine vapours as the locating agent.

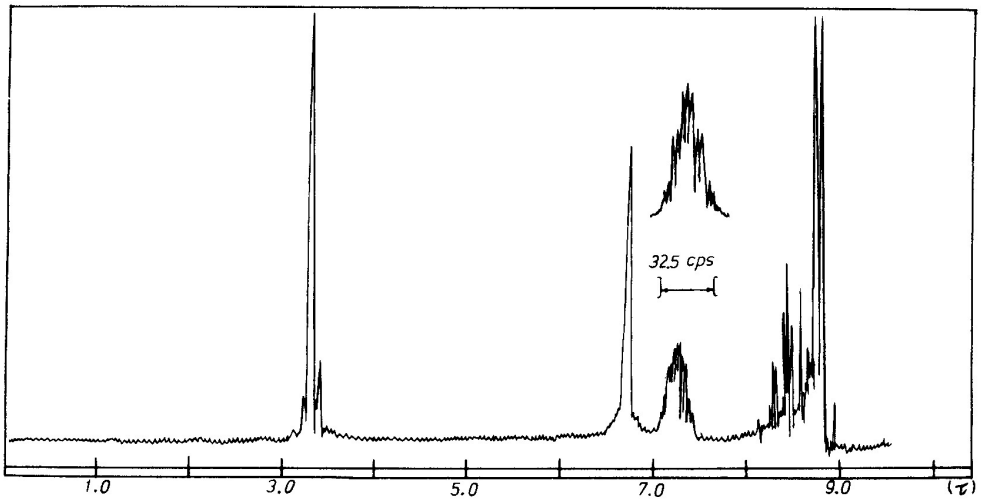


FIGURE 2—Pmr spectrum of compound (I) in $CDCl_3$.

If compound (I) were a mixture of the cis and trans isomers, the ring deshielding of the equatorial methine proton of the latter isomer should have assigned another low-field multiplet (13). This effect would have been shown up, along with the absorption cited above, in the form of two separate multiplets, or as a single, much broader than 32.5 cps, distorted multiplet. As neither of these speculations was realized in the spectrum, the existence of a single cis form is once more established.

Pharmacology

The LD_{50} of compound (I), as assessed by the intraperitoneal injection in mice, is found as 800 mg per kg animal body weight. Toxic effects of the investigated compound were manifested in the form of severe convulsions, that were soon followed by death. When injected alone in mice, compound (I) in a dose of 200 mg per kg body weight brings about a marked tranquilizing effect, as it was shown by quiescence of the animals in comparison with the control set. Meanwhile, mice receiving 125 mg (per kg animal body weight) pentothal sodium in combination with a dose of 100 mg per kg body weight of the investigated compound were noticeably affected, as reflected by the sleeping (anaethesia) time of this group (~15 minutes), and that receiving pentothal sodium alone (~5 minutes). Higher doses of compound (I) did not enhance considerably further potentiation of this barbiturate derivative, while doses lower than 100 mg showed moderate activity.

The intravenous administration of 5 mg of compound (I) per kg rabbit body weight revealed a generalized decrease in the mean amplitude of the biopotentials, recorded from all areas under test. Comparison with normal levels was assessed via tracing of the changes in the EEG patterns, Fig. 3.

A considerable decrease in the theta and alpha waves, in addition to an increase in the fast activities, can be easily recognized as well. Such changes were manifested after 15 minutes of administration, and were drawn back to normal within 2.5 hr. In addition, no effect on the phonic or photic reaction times was developed. Such changes are quite similar to those demonstrated by the common tranquilizer diazepam

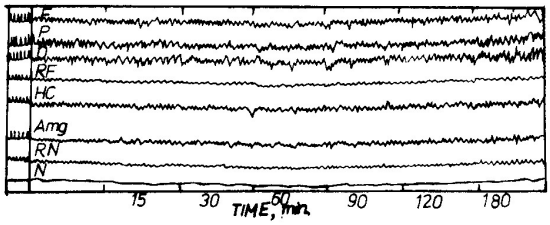


FIGURE 3—EEG patterns under the influence of compound (I) on rabbit, F = frontal area, P = parietal area, O = occipital area, RF = reticular formation, HC = hippocampus, Amg = amygdala, RN = red nucleus, and N = normal.

(14). The detailed study of the pharmacologic effect of compound (I) is in progress.

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