PRODUCTS OF THE ACID HYDROLYSIS OF 7-CHLORO-1,3-DIHYDRO-3-HYDROXY-5-PHENYL-2*H*-1,4-BENZODIAZEPIN-2-ONE (OXAZEPAM)

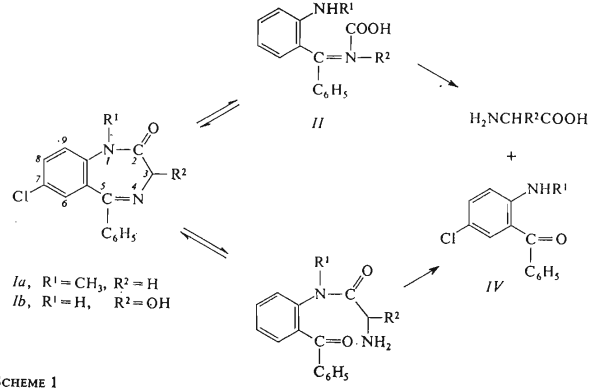
Jiří Gasparič[a], Jiří Zimák[a], Petr Sedmera[b], Zdeňka Breberová[a] and Jiří Volke[c]

[a] *Faculty of Pharmacy, Charles University, 501 65 Hradec Králové,*
[b] *Institute of Microbiology,*
Czechoslovak Academy of Sciences, 142 20 Prague 4 and
[c] *J. Heyrovský Institute of Physical Chemistry and Electrochemistry,*
Czechoslovak Academy of Sciences, 118 40 Prague 1

Received May 25th, 1978

The acid hydrolysis of oxazepam (*Ib*) was studied. Under various reaction conditions 6-chloro-4-phenylquinazoline-2-carbaldehyde (*V*) and the compounds *VI* and *VIII* were identified among the products besides the expected 2-amino-5-chlorobenzophenone (*IVb*).

The 1,4-benzodiazepine derivatives undergo easily hydrolysis in acidic media[1]. With diazepam *Ia* and oxazepam *Ib*, the hydrolysis of the 1,2-amidic and the 4,5-azomethine bonds[2] takes place in the first stage giving the intermediates *II* and *III* (Scheme 1). The final products of the decomposition are the corresponding 2-amino-



SCHEME 1

-5-chlorobenzophenones *IV* and the glycine derivatives. The reaction is considered as quantitative and since the resulting 2-aminobenzophenones are more suitable for analytical evaluation, the reaction is used for analytical purposes. The hydrolysis of oxazepam has been used for sensitive detection by means of colour reactions[3], before the thin-layer chromatography[4-7] or for photometric determination[8-10]. Also for the identification and quantitation by gas chromatography it is recommendable to convert oxazepam to 2-amino-5-chlorobenzophenone[11,12], since oxazepam itself is unstable at elevated temperatures[13-16]. Recently, the hydrolysis of oxazepam has been employed before high-pressure liquid chromatography[17]. In most cases the authors used 3–6M-H_2SO_4 or 6M-HCl as the reagent. In this communication we studied the hydrolysis of oxazepam under various conditions.

The semiquantitative experiments were performed in two series with diazepam *Ia* and oxazepam *Ib*. The products were detected by thin-layer chromatography. Whereas with diazepam the benzophenone *IVa* was the only product under all

TABLE I

Semiquantitative Evaluation of the Products of Oxazepam Hydrolysis After Chromatographic Analysis

Reaction time 180 minutes. The acid to alcohol ratio in the mixtures was 1 : 9. Symbols: – not present, + very little, ++ small amount, +++ large amount.

Medium	*V*	*IV*	*VI*	*VIII*	Medium	*V*	*IV*	*VI*	*VIII*
50% H_2SO_4	+	–	–	–	5% H_2SO_4	–	+	+	–
+ methanol	+	++	+	–	+ methanol	+	+++	+	+
+ ethanol	++	+	+	+	+ ethanol	+++	++	+	++
+ 2-propanol	+	+	++	++	+ 2-propanol	++	++	++	+
50% H_3PO_4	–	+	–	+	5% H_3PO_4	+	+	+	–
+ methanol	+++	+++	++	+	+ methanol	+++	+	–	+
+ ethanol	++	++	+	+	+ ethanol	+++	+	+	+
+ 2-propanol	++	+++	++	+	+ 2-propanol	++	+	+	–
98% CH_3COOH	++	+	+	–	50% CH_3COOH	++	+	+	–
+ methanol	++	–	–	–	+ methanol	+++	–	++	–
+ ethanol	+++	–	+	–	+ ethanol	+++	–	+	–
+ 2-propanol	++	–	+	–	+ 2-propanol	++	–	+	–
37% HCl					17% HCl	–	+++	++	–
+ methanol	++	++	++	–	+ methanol	+	+++	+	–
+ ethanol	++	+	+++	+	+ ethanol	++	+	+++	++
+ 2-propanol	++	+	++	++	+ 2-propanol	++	+	+++	+++

conditions studied, with oxazepam the sought 2-amino-5-chlorobenzophenone was not the main product in any case and three other compounds were formed in non-negligible amounts. Our attention was directed to the search of the conditions for the favoured formation of the individual hydrolysis products to facilitate their isolation and identification. The results of acid hydrolysis of oxazepam in the presence of sulfuric, hydrochloric, phosphoric, and acetic acids are summarized in Table I.

It can be seen that three further compounds were found in significant amounts besides the unreacted oxazepam and the expected benzophenone on chromatograms (Silufol/benzene–acetone 4 : 1 and Silufol/chloroform–benzene 2 : 1).

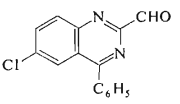
The yellow spot with R_F 0·80 and 0·40, respectively, was due to the 2-amino--5-chlorobenzophenone (*IVb*) which was also identified by mixed melting point, ^{1}H-NMR spectrum and mass spectrum.[18]

Compound with R_F 0·58 and 0·08, respectively, reacts with 2,4-dinitrophenylhydrazine. It was identified as 6-chloro-4-phenylquinazoline-2-carbaldehyde (*V*) which is formed from oxazepam by boiling with acetic acid[19] or by its thermal decomposition[14]. Its identity was confirmed by melting point, mass, ^{1}H- and ^{13}C--NMR spectra.

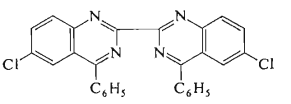
The spot on the thin-layer chromatogram corresponding to the compound X (R_F 0·65 and 0·05, respectively) exhibits a deep violet fluorescence in the UV-light (254 nm). That of compound Y (R_F 0·90 and 0·72, respectively) was yellow with yellow fluorescence in UV-light (254 nm). Compound X was the prevailing product of the hydrolysis in ethanolic hydrochloric acid. It differred from the other reaction products by its poor solubility and was therefore isolated by crystallization. Compound Y was isolated after hydrolysis in the mixture of concentrated hydrochloric acid and methanol (3 : 7) using chromatography on the layers of aluminium oxide.

Compound X has the elemental composition $C_{28}H_{16}Cl_2N_4$ (high resolution MS), indicating its dimeric character. The ion m/z 77 evidences the presence of a phenyl group. There are no NH, OH or CO bands in the infrared spectrum. The ^{1}H-NMR spectrum contains the signals of aromatic protons only. Eleven signals observed in the ^{13}C-NMR spectrum indicate a certain symmetry of the molecule. According to their chemical shifts, all carbons belong to the sp^2-type. Using the gated decoupling experiment which provides the noise decoupled ^{13}C-NMR spectrum without nuclear Overhauser effect[20], it was found that the signal δ_C 130·1 corresponds to three and that with δ_C 128·5 to two carbon atoms. The signals of double intensity are readily assigned to the *ortho-* and *meta*-carbons of the phenyl group. From an off-resonance experiment it follows that six signals are singlets (quaternary carbons) and five are doublets (methines). A comparison with ^{13}C-NMR of the aldehyde *V* allows to assign the three most lowfield signals (δ_C 168·7, 159·2, 150·1) to the carbons of the —N=C—N and —N—C= (2×) type in the quinazoline ring. This conclu-

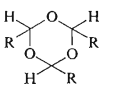
sion is confirmed also by comparison with the data for quinazoline[21]. The remaining carbon atoms might be assigned to the chlorine-substituted carbons of the substituted ring and to the 4'-carbon of the phenyl group. Therefore, the compound X is 2,2'--bis(6-chloro-4-phenylquinazolyl) (*VI*). Caille and coworkers[22] described a compound melting at 312 ± 3° whose infrared spectrum is practically identical with that of X, however, they assigned to this compound formed in the fluorometric determination of oxazepam in phosphoric acid the structure *VII*.

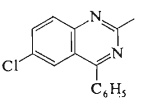


V

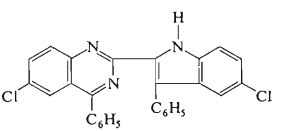


VI





VII



VIII

Compound Y has the elemental composition (high resolution MS, m/z 466) $C_{28}H_{17}Cl_2N_3$. The molecular weight determined by vapour pressure osmometry was 445·1. Therefore, this compound is also formed by a condensation of two oxazépam units. The ^{1}H-NMR spectrum contains a complex multiplet of aromatic protons and an isolated one-proton singlet at 10·08 ppm. The later signal is due to an exchangeable proton since its intensity decreases upon addition of CF_3COOD. The selective decoupling ^{13}C-{^1H} proves that this proton is not attached to any carbon. The ^{13}C-NMR spectrum contains 24 signals of the sp^2-type carbons. Four of them are of double intensity and can be assigned to the *ortho*- and *meta*-carbons of the two magnetically nonequivalent phenyl groups. According to an off-resonance experiment, there are 12 signals of quaternary carbon atoms and the rest are methine carbons. The three most lowfield signals (δ_C 166·7, 155·1, 150·1) can be similarly to the compound X assigned to a —N=C—N carbon and to the two carbons of the —C=N— type. This indicates the presence of a quinazoline nucleus. Since there are two chlorine atoms in the molecule, it is necessary to assign the two quaternary

carbon atoms to the carbons at the position 7 of the original ring. Two quaternary atoms are due to the carbons at the position 1′ and one another pair to that at the position 5a. One signal of the remaining three carbon atoms is shifted downfield what indicates a carbon attached to nitrogen. The above data are satisfied by the formula *VIII*.

Polarographic and spectrophotometric measurements of the pure components showed that the quantitative analysis of the five-component mixture was not possible without the use of separation techniques. As an example, we analysed the mixture formed by hydrolysis of oxazepam in ethanol and hydrochloric acid (9 : 1). The yield of the all products was in the range 45 – 50% with respect to the starting amount of oxazepam and was practically independent on the hydrolysis time. Thin-layer chromatography and the spectrophotometric determination showed that 50 – 52% of the isolated products was the compound X and 36 – 38% was the compound Y, *i.e. c.* 25% and 16% of the total yield. The study of the time dependence of the reaction mixture composition showed that the first product is the aldehyde *V* which disappears during the further reaction and the amount of the compounds X, Y, and benzophenone increases. The hydrolysis of the authentic aldehyde yields practically the same products. Compounds X and Y are however stable even after ten hours' boiling. The contraction of the seven-membered ring to the six-membered one was already described during the acid hydrolysis of 1,4-benzodiazepines[23,24]. However, the formation of dimers and the contraction to a five-membered ring has not been observed yet.

EXPERIMENTAL

The melting points were determined in a Kofler hot stage and were corrected. Thin-layer chromatography was performed on Silufol® sheets (Kavalier, Votice, Czechoslovakia) in the systems chloroform–benzene (2 : 1) or benzene–aceton (4 : 1). Spots were detected in the UV-light at 254 nm (Universal UV-Lampe Camag, Mutenz). The UV spectra were measured on the Unicam SP 1800 spectrophotometer. IR spectra were measured on the Perkin–Elmer 577 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured on the Jeol FX – 60 (59·797 and 15·036 MHz) spectrometer in hexadeuteriodimethyl sulfoxide whose central signal δ_C 40·4 was used as an internal reference. The mass spectra were recorded on the Varian MAT 311 instrument. For vapour pressure osmometry the Knauer osmometer was used and the determination was made in benzene.

Oxazepam used in all experiments (Léčiva, Dolní Měcholupy, Czechoslovakia) was chromatographically pure. Mass spectrum *m/z* (% of relative intensity): 231 (72·5), 230 (100), 214 (6), 195 (21), 154 (23), 126 (15), 105 (29), 77 (40), 36 (63).

6-Chloro-4-phenylquinazoline-2-carbaldehyde (*V*)

Oxazepam (10 g) and glacial acetic acid (5 ml) were boiled under reflux 10 minutes. After cooling, the mixture was diluted with water, the precipitate was filtered off and dried. The crude product was dissolved in hot ethanol, the solution was boiled with active charcoal, filtered and then water

was added until first opalescence was observed. After 24 hours the crystals were filtered off and dried. They melted at 176—178°C (lit.[14] gives 177—178°C. Mass spectrum m/z (% of relative intensity): 268 (78, M), 267 (66), 239 (65), 233 (76), 205 (76), 177 (25), 151 (18), 104 (27), 77 (100), 51 (37). ^{13}C-NMR δ_C, off-resonance multiplicity: 191·4 d, 168·9 s, 155·0 s, 149·6 s, 136·2 s, 135·4 d, 131·6 d, 130·6 d, 129·9 d (2C), 128·8 d (2C), 125·9 d, 123·8 s.

Semiquantitative Hydrolysis of Oxazepam

Oxazepam (0·1 g) was boiled under reflux with 5 ml of the mixture of the solvent and acid for 3 hours (Table I). Samples were taken after 5, 10, 15, 30, and 180 minutes and subjected to chromatography in the system benzene–chloroform (2 : 1) together with the standards.

Isolation of (2,2′-Bis(6-chloro-4-phenylquinazolyl) *VI*, Compound X

Oxazepam (2 g) was refluxed in the mixture (20 ml) of ethanol and hydrochloric acid (9 : 1) for one hour. After cooling the reaction mixture was ammonia neutralized and the precipitate was filtered off. Crystallization from toluene yielded needles m.p. 316°C (yield 0·6 g). The compound is soluble in chloroform and in hot toluene. ^{1}H-NMR: 7·73—8·72 mt. ^{13}C-NMR δ_C, off-resonance multiplicity: 168·7 s, 159·2 s, 150·1 s, 136·6 s, 134·6 d, 134·1 s, 131·5 d, 130·1 d (3C), 128·5 d (2C), 125·7 d, 122·9 s. Mass spectrum m/z (% of relative intensity, composition): 479· (80), 478 (67), 477 (100, $C_{28}H_{15}Cl_2N_4$), 443 (60, $C_{28}H_{16}ClN_4$), 177 (13, $C_{13}H_7N$), 151 (10, $C_{12}H_7$), 77 (52, C_6H_5).

Isolation of 2-(5-Chloro-3-phenyl-2-indolyl)-6-chloro-4-phenylquinazoline (*VIII*), Compound Y

Oxazepam (1·5 g) and 10 ml of the mixture of methanol and concentrated hydrochloric acid (7 : 3) were boiled under reflux for 4 hours. The precipitate isolated as above was dissolved in hot benzene and separated by thin-layer chromatography on aluminium oxide. The chromatograms were developed with benzene and the spots were detected in the UV light. The zone containing compound Y was removed and eluted with benzene. After concentrating the solution, yellow crystals m.p. 201°C (yield 20 mg) were obtained. This compound is good soluble in organic solvents. Mass spectrum m/z (% of relative intensity, elemental composition): 467 (72), 466 (70), 465 (100, $C_{28}H_{15}Cl_2N_3$), 464 (64), 428 (12, $C_{28}H_{15}ClN_3$), 388 (10, $C_{22}H_{12}ClN_3$), 361 (3·5, $C_{21}H_{11}Cl_2N_2$), 352 (3·5), 326 (9), 77 (10·5), 36 (17). ^{1}H-NMR δ_H, multiplicity: 7·40—8·46 mt (16 H), 10·08 br s (1 H). ^{13}C-NMR δ_C, off-resonance multiplicity: 166·7 s, 155·1 s, 150·1 s, 136·3 s, 134·5 s, 134·4 d, 134·0 s, 132·5 s, 131·9 s, 131·0 d (2 C), 130·6 s, 130·2 d (2 C), 130·1 d (2 C), 128·4 d (2 C), 127·6 d (2 C), 126·7 d, 126·1 s, 125·7 d, 124·8 d, 121·8 s, 120·9 s, 120·3 d, 112·2 d.

Quantitative Study of the Oxazepam Hydrolysis

Oxazepam (0·1 g) was boiled under reflux with 5 ml of the mixture of ethanol and hydrochloric acid for 1, 4, and 6 hours. Two paralel experiments were made in each case. Completing the boiling, the mixture was neutralized with ammonia, the precipitate was filtered off and dried to the constant weight. The total yields were 46 ± 2% (average of six determinations). The dried mixture (20 mg) was dissolved in chloroform (10 ml) and 25 µl of this solutions was applied in the form of a narrow streak on a Silufol plate. The chromatogram was developed with benzene and chloroform (2 : 1). The zones containing the compounds X and Y were after the detection (UV-lamp) eluted each by 10 ml of chloroform The absorbancy of these solutions was measured

at 260 nm (compound X) and at 256 nm (compound Y). Calibration curves were constructed for both components. All measurements were made against the blank, *i.e.* the eluate of the corresponding part of the Silufol sheet developed without any sample. The average of six determinations was 40·5% for X and 38·9% for Y, with respect to their content in the dried product of the hydrolysis.

REFERENCES

1. Sternbach L. H., Reeder R.: J. Org. Chem. *26*, 1111 (1961).
2. Han W. W., Yakatan G. J., Maness D. D.: J. Pharm. Sci. *66*, 573 (1977).
3. Bohn G., Clasing D.: Sportarzt und Sportmedizin *2*, 31 (1972).
4. Schütz C., Schütz H.: Klin. Chem. Klin. Biochem. *10*, 528 (1972).
5. Kamm G., Kelm R.: Arzneim.-Forsch. *19*, 1659 (1969).
6. Tokunaga K.: Kagaku Keisatsu Kenkyusho Hokoku *23*, 253 (1970); Chem. Abstr. *75*, 32230 (1971).
7. Lafargue P., Meunier J., Lemontey Y.: J. Chromatogr. *62*, 423 (1971).
8. Walkenstein S. S., Wiser R., Gudmundsen C. H., Kimmel H. B., Corradino R. A.: J. Pharm. Sci. *53*, 1181 (1964).
9. Raber H., Gruber J.: Sci. Pharm. *40*, 35 (1972).
10. Amal H., Ates O.: Istanbul Univ. Eczacilik Fak. Mecm. *9*, 37 (1973); Chem. Abstr. *81*, 82455 (1974).
11. Knowles J. A., Ruelius H. W.: Arzneim.-Forsch. *22*, 687 (1972).
12. Vessman J., Freij G., Stromberg S.: Acta Pharm. Suec. *9*, 477 (1972).
13. Sadée W., van der Kleijn E.: J. Pharm. Sci. *60*, 135 (1971).
14. Forgione A., Martelli P., Marcucci F., Fanelli R., Mussini E., Jommi G. C.: J. Chromatogr. *59*, 163 (1971).
15. De Meijer P. J. jr, J.: Pharm. Weekbl. *108*, 849 (1973).
16. Frigerio A., Baker K. M., Belvedere G.: Anal. Chem. *45*, 429 (1973).
17. Harzer K., Barchet R.: J. Chromatogr. *132*, 83 (1977).
18. Rendić S., Klasinc L, Šunjić. V., Kajfež F., Kramer V., Mildner P.: Biomed. Mass Spectrometry *2*, 97 (1975).
19. Bell S. C., Childress S. J.: J. Org. Chem. *29*, 506 (1964).
20. Feeney J., Shaw D., Pauwells P. J. S.: Chem. Commun. *1970*, 554.
21. Stothers J. B.: *Carbon*-13 NMR *Spectroscopy*, p. 262. Academic Press, New York, 1972.
22. Caille G., Braun J., Gravel D., Plourde R.: Can. J. Pharm. Sci. *8*, 42 (1973).
23. Carstensen J. T., Su K. S. E., Madrell P., Johnson J. B., Newmark H. N.: Bull. Parenteral. Drug. Assoc. *25*, 193 (1971).
24. Genton D., Kesselring U. W.: J. Pharm. Sci. *66*, 676 (1977).

Translated by the author (P. S.).