THE SYNTHESIS OF 6-AMINO-8-HYDROXY-4-OXO-2-(4-PENTYLOXYBENZOYL)-5,7-DIAZASPIRO[2.5]OCTA-5,7-DIENE-1-CARBOX-N-CYCLOPENTYLAMIDE*

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Condensation of diethyl 2-methoxycarbonyl-3-(4-pentyloxybenzoyl)-cyclopropane-1,1-dicarboxylate with guanidine gave methyl 6-imino-4,8-dioxo-2-(4-pentyloxybenzoyl)-5,7-diazaspiro[2.5]-octane-1-carboxylate (*I*) which was saponified to corresponding carboxylic acid *II*. The imidazolide of acid *II* reacted with cyclopentylamine to afford 6-amino-8-hydroxy-4-oxo-2-(4-pentyloxybenzoyl)-5,7-diazaspiro[2.5]octa-5,7-diene-1-carbox-N-cyclopentylamide (*III*). Compound *III* when applied *per os* prolonged the life of animals with some transplanted tumours.

In connection with the study of the relationships between the structure of substances and their antineoplastic effect in animals with experimental tumors we also investigated the synthesis of 6-amino-8-hydroxy-4-oxo-2-(4-pentyloxybenzoyl)-5,7-diazaspiro[2.5]octa-5,7-diene-1-carbox-N-cyclopentylamide (*III*).

Condensation of diethyl ester of 2-methoxycarbonyl-3-(4-pentyloxybenzoyl)-cyclopropane-1,1-dicarboxylic acid[1] with guanidine in the presence of sodium methoxide in boiling methanol gave methyl 6-imino-4,8-dioxo-2-(4-pentyloxybenzoyl)-5,7-diazaspiro[2.5]-octane-1-carboxylate (*I*) which on alkaline saponification afforded 6-imino-4,8-dioxo-2-(4-pentyloxybenzoyl)-5,7-diazaspiro[2.5]-octane-1-carboxylic acid (*II*). On reaction with N,N'-carbonyldiimidazole acid *II* was converted to corresponding imidazolide, which was reacted with cyclopentylamine to give 6-amino-8-hydroxy-4-oxo-2-(4-pentyloxybenzoyl)-5,7-diazaspiro[2.5]-octa-5,7-diene-1-carbox-N-cyclopentylamide (*III*).

The structures of compounds *I*–*III* were confirmed by their IR and UV spectra, and in the case of compound *I* also by its mass spectrum. In the spectra of compounds *I* and *II* the typical bands in the $—NH_2$ absorption region were absent, while the bands of —NH— or =NH bonds in the 3340 cm^{-1} region were present. In the 1500–1800 cm^{-1} region the course of the spectra of compounds *I* and *II* is similar. In the case of compound *I* the sharp band in the ester carbonyl region occurs at 1740–1755 cm^{-1}, which is substituted by the carboxyl group band at 1700 cm^{-1}

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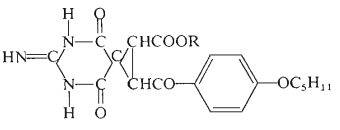
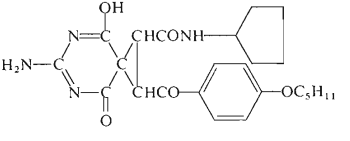


I, R = CH_3
II, R = H



III

in the case of compound *II*. In both cases a maximum at 1715–1725 cm^{-1} is present which we assign to the cyclic amide, and the band at 1640 cm^{-1} due most probably to the C=N-bond. In contrast to this in the case of substance *III* no band is present in the region of cyclic amide (1715–1725 cm^{-1}), while in the —NH-bonds region two sharp absorption maxima occur corresponding to a $-NH_2$ group, and a broad maximum appears in the 3100–3200 cm^{-1} region, indicating the presence of —OH bonds. In addition to the intensive band at 1640 cm^{-1} (C=N) further maxima appear at 1660 cm^{-1} (amide *I*) and at 1580 (amide II), which we assign to the carboxamide group. From the presented data we judge that the structures of compounds *I* and *II* are similar, while we assign to compound *III* (in solid state) the given tautomeric form of the heterocyclic part of the molecule. The presence of the conjugated CO-group in compounds *I*–*III* was demonstrated both by the absorption band in the IR region, occurring at 1670–1685 cm^{-1}, and by an identical course of absorption curves in the UV region, with a practically identical value of the molecular extinction coefficient of the absorption maximum at 285–290 nm (log ε = 4·25 to 4·28).

The preliminary testing of compounds *I*–*III* on their antineoplastic effect in animals with transplanted tumours was carried out by Dr K. Řežábek and coworkers of our Institute. The substances were administed *per os* to mice of strain H with ascitic tumour 37 (S 37) in a 100 mg/kg dose per day over a period of 8 days, beginning with the 3rd day from the implantation of the tumour cells, and in the same dose and using the same method of application to Wistar rats Yoshida ascitic sarcoma(Y), for 5 days from the 2nd day of the implantation of the tumour. Substance *III* prolonged the time of survival of experimental animals with the tumour S 37 for 33%, without affecting the size of the tumour, and in rats with tumour Y for 22% (in comparison with the control group of non-treated animals). More detailed data on the biological evaluation of the substances will be published elsewhere.

EXPERIMENTAL

The melting points were determined on a Kofler block. Samples for analysis were dried at proportionally elevated temperature in a vacuum 0·1 Torr. The purity of the substances was checked by chromatography on Silufol UV_{254} (Kavalier, Czechoslovakia), using a benzene–methanol 9 : 1 mixture for development. The IR spectra were recorded on an Infrascan instrument

from Hilger–Watts, London, in KBr pellets (2·5 mg/700 mg of KBr). The ultraviolet spectra were recorded with an Optica, Milano CF4R instrument in 10 mm quartz cells (0·001% in ethanol).

Methyl 6-Imino-4,8-dioxo-2-(4-pentyloxybenzoyl)-5,7-diazaspiro[2.5]octane-1-carboxylate (*I*)

Guanidine hydrochloride (2·23 g; 0·023 mol) and a solution of 10 g (0·023 mol) of diethyl ester of 2-methoxycarbonyl-3-(4-pentyloxybenzoyl)cyclopropane-1,1-dicarboxylic acid[1] in 80 ml methanol were added to a solution of 1·08 g (0·047 mol) of sodium in 150 ml of methanol. The mixture was refluxed for 19 h, diluted with 270 ml of methanol and filtered with charcoal. The filtrate was evaporated and the residue (13·4 g) dissolved in 10 ml of methanol and the solution diluted with 900 ml of ether. After 2 h standing at 0°C the separated product was filtered off under suction dissolved in 30 ml of the mixture chloroform–methanol (1 : 1) and the filtrate evaporated. The residue (8·1 g, 87%) was purified by chromatography on a silica gel column (50 g) using chloroform–methanol mixture (1 : 1), or pure methanol for the elution of end fractions, as eluent. Combination of corresponding fractions gave 3·5 g (38%) of a compound that was recrystallized from aqueous methanol for analysis. If heated above 200°C the compound darkens without melting previously. For $C_{20}H_{23}N_3O_6$ (401·4) calculated: 59·83% C, 5·77% H, 10·47% N; found: 59·47% C, 5·69% H, 10·29% N. IR spectrum: 3340 (=NH), 2780–2920 (—OC_5H_{11}, —CH), 1740–1755 (ester CO), 1720 (cyclic amide), 1686 (aryl-CO), 1640 (C=N), 1610, 1505, and 845 cm^{-1} (*p*-substituted aromatic). Mass spectrum: *m/e* 401, 1566, corresponds to the composition $C_{20}H_{23}N_3O_6$, and in agreement with the structure the following abundant fragment peaks were also found: *m/e* 271·0588 ($C_{13}H_9N_3O_4$), 191·1078 ($C_{12}H_{15}O_2$) and 121 ($C_7H_5O_2$, and less intensive fragments *m/e* 210 ($C_8H_8O_4N_3$) and 94 ($C_5H_2O_2$).

6-Imino-4,8-dioxo-2-(4-pentyloxybenzoyl)-5,7-diazaspiro[2.5]octane-1-carboxylic Acid (*II*)

A solution of 3·7 g (0·009 mol) of compound *I* in 75 ml of 1M-NaOH was allowed to stand at 20°C, for 2 h, then filtered and acidified with hydrochloric acid to Congo Red. After 2 days, standing at 0°C the product was filtered off under suction, dissolved in dimethylformamide, the solution diluted with 25 ml of water and set aside at 0°C. The obtained suction-dried substance was further dried at 60°C/12 Torr (it weighs 3·4 g; 95%), m.p. 249–251°C. For analysis the substance was repeatedly crystallized from aqueous dimethylformamide, m.p. 252–253°C. For $C_{19}H_{21}N_3O_6$ (387·4) calculated: 58·90% C, 5·46% H, 10·84% N; found: 58·50% C, 5·63% H, 10·89% N. IR spectrum: 3342 (=NH), 2780–2925 (OC_5H_{11}, —CH), 1718 (cyclic amide), 1700 (COOH), 1680 (aryl-CO), 1638 (C=N), 1612, 1508, and 848 cm^{-1} (*p*-substituted aromate).

6-Amino-8-hydroxy-4-oxo-2-(4-pentyloxybenzoyl)-5,7-diazaspiro[2.5]octa-5,7-diene-1-carbox-N-cyclopentylamide (*III*)

N,N'-Carbonyldiimidazole (0·54 g; 0·003 mol) was added to a solution of 1·14 g (0·003 mol) of compound *II* in 30 ml of dimethylformamide at 20°C and the mixture allowed to stand at 20°C for 2 h under exclusion of air humidity. Cyclopentylamine (0·6 ml; 0·007 mol) was then added and the mixture allowed to stand at 20°C for 6 days. The solvent was evaporated and the residue stirred with 15 ml of water and set aside overnight (at 3°C). The product was filtered off under suction (1·45 g) and crystallized from chloroform–methanol (0·66 g, 49%), m.p. 250–255°C. A sample for analysis was repeatedly crystallized from the same solvent mixture, m.p. 275–277°C. For $C_{24}H_{30}N_4O_7$ (454·5) calculated: 63·41% C, 6·65% H, 12·32% N; found: 63·03% C, 6·74% H,

12·24% N. IR spectrum: 3360, 3280 (—NH_2), 3120 (—OH), 2910—2720 (—OC_5H_{11}, —CH, —CH_2), 1660—1680 (aryl-CO, amide I), 1635 (C=N), 1620, 1585 (aromatic, amide II), 1505, 825—840 cm^{-1} (*p*-substituted aromatic).

The analyses of substances mentioned in this paper were carried out in this analytical department of our Institute by Mrs J. Komancová, under the direction of Dr J. Körbl; for the measurement of the mass spectra our thanks are due to Dr M. Ryska of our Institute.

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