SUBSTANCES WITH ANTINEOPLASTIC ACTIVITY. LII.*

SOME β-AMINO-SUBSTITUTION DERIVATIVES OF γ-METHOXY-α-CHLORO(BROMO)-$\Delta^{\alpha,\beta}$-CROTONOLACTONE

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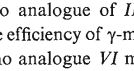
Reaction of γ-methoxy-α,β-dichloro-$\Delta^{\alpha,\beta}$-crotonolactone (*V*) or of its α,β-dibromo analogue *VI* with the corresponding amines produced γ-methoxy-β-diethylamino-α-chloro-$\Delta^{\alpha,\beta}$-crotonolactone (*I*), the analogous β-tetramethylenamino and β-pentamethylenamino compounds *II* and *III* and the α-bromo analogue of *III*, designated as *IV*. Reaction of lactone *V* with piperidine resulted at the same time in the pentamethylenamide of 2-pentamethylenamino-3-chloro-3-formylacrylic acid (*VII*). Substitution at the C_β of lactones *V* and *VI* with the residue of the amines used did not result in antineoplastically more active compounds.

In connection with studying the cytostatically effective γ-substituted α,β-dihalogeno-$\Delta^{\alpha,\beta}$-crotonolactones and related compounds[1-3] we prepared β-diethylamino-, β-tetramethylenamino- and β-pentamethylenamino-γ-methoxy-α-chloro-$\Delta^{\alpha,\beta}$-crotonolactones (*I*–*III*) and the α-bromo analogue of *III*, designated here as *IV*. It was of interest to see to what extent the efficiency of γ-methoxy-α,β-dichloro $\Delta^{\alpha,\beta}$-crotonolactone (*V*) and of its α,β-dibromo analogue *VI* might be affected by replacing the halogen at C_β with a secondary amine residue.



I; $R^1 = Cl$, $R^2 = N(C_2H_5)_2$
II; $R^1 = Cl$, $R^2 = NCH_2(CH_2)_2CH_2$
III; $R^1 = Cl$, $R^2 = NCH_2(CH_2)_3CH_2$
IV; $R^1 = Br$, $R^2 = NCH_2(CH_2)_3CH_2$
V, $R^1 = R^2 = Cl$
VI; $R^1 = R^2 = Br$

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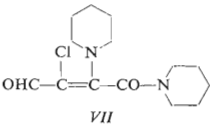
Compounds *I-III* and *IV* were obtained in a fine yield by a reaction of lactones *V* and *VI* which can be also formulated as methyl esters of the cyclic form of mucochloric and mucobromic acids with two equivalents of the corresponding amines, in benzene at reduced temperature. The reaction course was analogous as in the case of the reaction of these lactones with aniline as described by Wasserman and Precopio[4]. The reaction of lactone *V* with piperidine led at the same time to the pentamethylenamide of 2-pentamethyleneamino-3-chloro-3-formylacrylic acids (*VII*) (12%) which was prepared by Winterfeldt and Nelke[5] by the reaction of α,β,γ-trichloro-$\Delta^{\alpha,\beta}$-crotonolactone with piperidine. The methoxylactone *V* and the abovementioned trichloro compound, the chloride of the cyclic form of mucochloric acid, react with piperidine, giving rise to amide *VII*, apparently in an analogous way. Lactones *V* and *VI* were prepared with advantage by the reaction of a mucohalogenic acid with methanol at 60°C, in the presence of anhydrous zinc chloride, in a yield of 85 and 58%, respectively.



VII

Compounds *I–IV* show in the IR region a maximum of the lactone carbonyl at 1745–1755 cm^{-1} which indicates the presence of halogen at the C_α of the molecule. The presence of halogen at C_β of compounds of the same type[1] appears to cause a shift of the carbonyl maximum to the region of 1770–1780 cm^{-1}, both in a chloroform solution and in a KBr pellet.

The absorption maximum of the —C=C— bond of *I–IV* lies at 1625–1630 cm^{-1}, that of the methoxy group at 1122 and 2848 cm^{-1}. Compound *VII* shows absorption maxima at 2680–2780 cm^{-1} which indicates the presence of an aldehyde group in the molecule, the maximum at 1642 cm^{-1} corresponds to an aldehyde carbonyl and that at 1552 cm^{-1} to an amidic bond. The shown structures of *I–IV* and *VII* also agree with their UV and NMR spectra.

An informative evaluation of *I–VI* as to their antineoplastic effect in mice of the H strain and in Wistar rats with transplantable tumours was carried out at this institute by Dr V. Jelínek and Dr H. Veselá with coworkers (in H-strain mice: Crocker's sarcome 180–S180, mammary adenocarcinome — HK, S37 sarcome; in rats: Yoshida's ascitic sarcome — Y; for the technique and evaluation of results see ref.[6]). The compounds were applied per os at a daily dose of 100 mg/kg, beginning on the third day (with animals bearing S180 and HK) or on the second day (S37 and Y) after tumour transplantation, continuously for 12 days, with the exception of animals bearing Y, where the compounds were applied for only 5 days. Rats with the Y tumour were given the compounds also intraperitoneally, at a daily dose of 20 mg/kg, otherwise under the same conditions as shown for the *p.o.* application to animals with the Y tumour.

A more pronounced therapeutical effect was shown only by *IV* and *VI*, administered *i.p.* to rats with the Y tumour. In comparison with the control group, the animals survived on compound *VI* by 71% and on compound *IV* by 54% longer. In summary, it can be said that replacement of the halogen at C_β of γ-methoxy-α,β-dichloro(dibromo)-$\Delta^{\alpha,\beta}$-crotonolactone with a residue of the secondary amines used did not result in antineoplastically more active substances.

EXPERIMENTAL

The melting points of the compounds were estimated in Kofler's block and are not corrected. Samples for analysis were dried *in vacuo* (0·1 Torr) at a temperature proportional to their melting point. The purity of the compounds and the course of column chromatography were checked by paper chromatography in formamide–phosphoric acid as the stationary phase and in benzene–cyclohexane (7 : 3) as the mobile phase. The compounds were detected on the basis of their fluorescence in UV light. The IR spectra were registered in a KBr pellet (2 mg compound per 600 mg KBr) in a UR-20 (C. Zeiss, Jena) spectrophotometer. The UV spectra were measured in methanol (0·5 mg%, 1 cm cuvette) on an Optica Milano CF 4R spectrophotometer. The NMR spectra were measured in $CDCl_3$, at a 6% concentration and using tetramethylsilane as standard, on a ZKR-60 (Zeiss, Jena) spectrometer.

γ-Methoxy-β-diethylamino-α-chloro-$\Delta^{\alpha,\beta}$-crotonolactone (*I*)

A solution of 14·6 g (0·2 mol) diethylamine in 300 ml benzene was added dropwise under stirring at 3°C over a period of 45 min to a solution of 18·3 g (0·1 mol) lactone *V* in 500 ml benzene, and after 1 h of stirring at 3°C, the mixture was left to stand overnight at 5°C. It was then extracted with water, the aqueous phase with benzene (2 . 100 ml) and the combined organic extracts were dried with Na_2SO_4 and the volatile components were removed by distillation in a water-pump vacuum. A solution of the residue in benzene was chromatographed on a column of silica gel (155 g, grain size 0·05—0·1 mm) in benzene. A total of 13·4 g (73%) lactone *I* was obtained; m.p. 45—47°C, after crystallization from ether m.p. 51—52°C. For $C_9H_{14}ClNO_3$ (219·7) calculated: 49·21% C, 6·42% H, 6·38% N, 16·14% Cl; found: 49·57% C, 6·51% H, 6·29% N, 16·09% Cl. UV spectrum: λ_{max} 284 nm (log ε 4·37). NMR spectrum: 5·65 p.p.m., s (1 H), 3·5 p.p.m., q (4 H), J = 7·0 Hz, 3·32 p.p.m., s (3 H), 1·22 p.p.m., t (6 H), J = 7·0 Hz.

Lactone II *or* IV was prepared in the same way as *I*, using the same molar ratios of lactone *V* or *VI* and of pyrrolidine or piperidine, as of the starting compounds. A total of 17·8 g (81%) crude substance (*II*) was obtained: m.p. 60—62°C, after crystallization from ether, m.p. 65—67°C. For $C_9H_{12}ClNO_3$ (217·7) calculated: 49·43% C, 5·99% H, 6·40% N, 16·21% Cl; found: 49·86% C, 5·62% H, 6·45% N, 16·17% Cl. UV spectrum: λ_{max} 288 nm (log ε 4·36). NMR spectrum: 5·60, p.p.m., s (1 H), 3·65 p.p.m., bs (4 H), 3·40 p.p.m., s (3 H), 1·60—2·20 p.p.m., m (4 H). 16·0 g (58%) of crude substance *IV* was obtained (m.p. 69—70°C); after crystallization from methanol it melted at 72—73°C. For $C_{10}H_{14}BrNO_3$ (276·1) calculated: 43·49% C, 5·11% H, 5·08% N, 28·94% Br; found: 43·35% C, 5·13% H, 5·22% N, 28·70% Br.

Lactone III *and amide* VII were prepared by using the same molar ratios of lactone *V* and piperidine and of the starting compounds, and the same conditions of condensation as in the case of preparation of lactone *I* from lactone *V* and diethylamine. Likewise, the processing of the reaction mixture was the same. During chromatography a total of 18·8 g crude lactone *III* was obtained and by subsequent elution with a mixture of benzene and 5% ethanol a total of 3·3 g (12%) crude amide *VII*. Crude lactone *III* was chromatographed on a column of alumina (130 g, activity II) in benzene. A total of 17·4 g (75%) product was obtained which was dissolved in a small amount of ether and cooled to —60°C to yield 13·9 g (60%) product, melting at 43—44°C. For $C_{10}H_{14}ClNO_3$ (231·7) calculated: 51·84% C, 6·09% H, 6·04% N, 15·34% Cl; found: 52·12% C,

6·14% H, 5·83% N, 15·54% Cl. UV spectrum: λ_{max} 284 nm (log 4·41). NMR spectrum: 5·65 p.p.m., s (1 H), 3·60 p.p.m., bs (4 H), 3·44 p.p.m., s (3 H), 1·67 p.p.m., bs (6 H).

Crude amide *VII* was crystallized from aqueous ethanol and ethyl acetate, m.p. 111—112°C (ref.[5] gives a m.p. of 112°C). UV spectrum: λ_{max} 319 nm (log ε 4·31), 254 nm (1·71). NMR spectrum: 9·15 p.p.m., s (1 H), 3·0—4·0 p.p.m., m (8 H), 1·30—2·0 p.p.m., bs (12 H).

γ-Methoxy-α,β-dichloro-$\Delta^{\alpha,\beta}$-crotonolactone (*V*)

A mixture of 16·9 g (0·1 mol) mucochloric acid, 40 ml methanol and 7·0 g anhydrous zinc chloride was heated for 7 h at 60°C. After addition of 50 ml water the product was extracted with benzene, the benzene extract was evaporated and the residue fractionally distilled. A total of 15·5 g (85%) lactone *V* was obtained, b.p. 108°C/11 Torr, n_D^{20} 1·4940 (ref.[7] gives a b.p. of 103°C/10 Torr, n_D^{20} 1·4932).

Lactone *VI*

A mixture of 150 g (0·57 mol) mucobromic acid, 93 ml methanol and 79 g anhydrous zinc chloride was heated for 1 h at 60°C, methanol was removed by distillation *in vacuo*. The residue was divided between 360 ml water and 600 ml chloroform, the organic phase was freed of the solvent by distillation and the residue recrystallized from methanol: 91·4 g (58%), m.p. 48·5—49°C (ref.[7] gives a m.p. of 48·5—49·5°C).

The analyses were done by Mr K. Havel and Mrs J. Komancová and Mrs V. Šmídová (headed by Dr J. Körbl) from the analytical department, the paper chromatography was performed by Mrs M. Jelínková, all of this Institute.

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