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Mass Spectra of Pyrimidines

Part II.* Structure Determination of 2,3-Dihydrothiazolo-[3,2-a]pyrimidinones

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A mass spectrometric method for the structure determination of isomeric 2,3-dihydrothiazolo[3,2-a]pyrimidinones is described. While the fused pyrimidines themselves are found unsuitable for detailed analysis, the mass spectra of the corresponding hydrolysis products, *i.e.* 1- and 3-(2-mercaptoethyl)uracils, permit unequivocal assignments of structure to the primary products.

Recently we described the mass spectrometric fragmentation of 1- and 3-alkyluracils.[1] We now report the application of the acquired knowledge to the structure determinations of isomeric 2,3-dihydrothiazolo[3,2-a] pyrimidinones (*e.g.* III and IV). A few derivatives of 2,3-dihydro-5*H*-thiazolo-[3,2-a]pyrimidin-5-one [2] and two derivatives of 2,3-dihydro-7*H*-thiazolo-[3,2-a] pyrimidin-7-one [3] have previously been described, but only in the latter case has the structure been firmly established by an unequivocal synthesis.

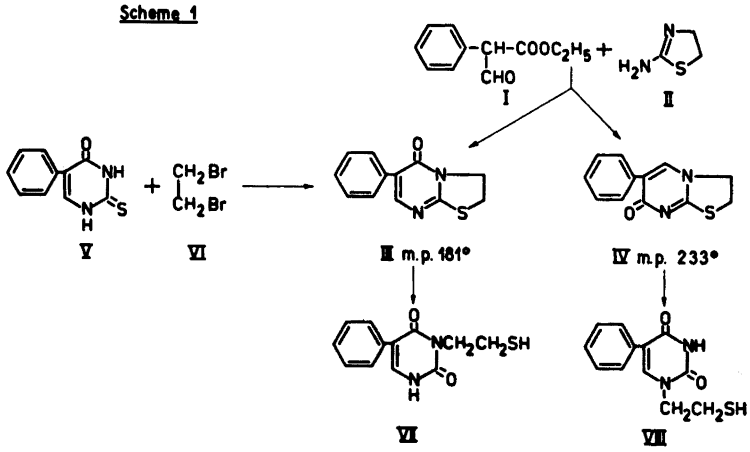
The simplest way of preparing the fused heterocycles of the above type is by treatment of β-oxocarboxylic acid derivatives with 2-amino-2-thiazolines. In this general reaction the formation of two isomeric compounds is of course always possible, and in some case both isomers can be isolated. The absence of clearly different structure elements in the reaction products (exemplified by III and IV) renders direct assignment of structure by conventional physical means (UV, IR, NMR, and MS) both difficult and uncertain. In the present work we wish to demonstrate how mass spectrometry of the hydrolysis products provides a rapid and convenient method for distinguishing between the two types of compounds.

When ethyl α-formylphenylacetate (I) and 2-amino-2-thiazoline (II) (Scheme 1) were refluxed in glacial acetic acid, a compound melting at 181° was isolated. On the other hand, the same reactants, mixed in ethanol at room temperature, gave an isomeric compound melting at 233°.

* For part I, see Ref. 1.

In the reaction of 5-phenyl-2-thiouracil (V) and ethylene bromide (VI) the major product was the compound melting at 181°, though a small amount of the isomer could be detected.

Scheme 1



The IR-spectra of the two compounds were almost identical while the UV-spectra were different. According to Allen *et al.*,[4] who assigned structures to thiazolo[3,2-a]pyrimidinones on the basis of UV-data, the compound melting at 181° should be III and the compound melting at 233° should be IV.

The mass spectra (see Experimental) of III and IV were almost identical; the most abundant ions were the molecular ion and an ion at *m/e* 102, probably phenylacetylene. Differentiation between the two structures was not possible at this stage. However, hydrolysis of III and IV in acid or base gave compounds with the isomeric structures VII or VIII, respectively. Analysis of their mass spectra (Figs. 1 and 2) permits assignment of structures to the precursors III and IV.

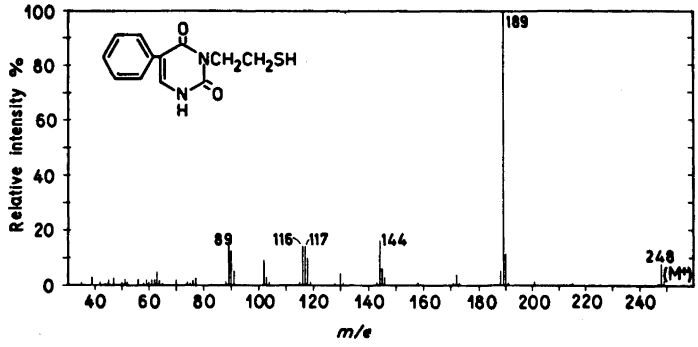


Fig. 1.

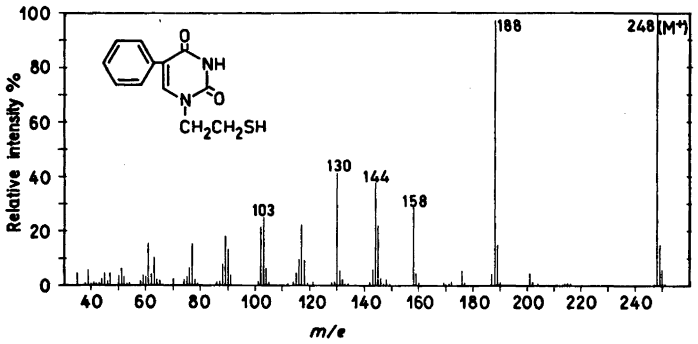
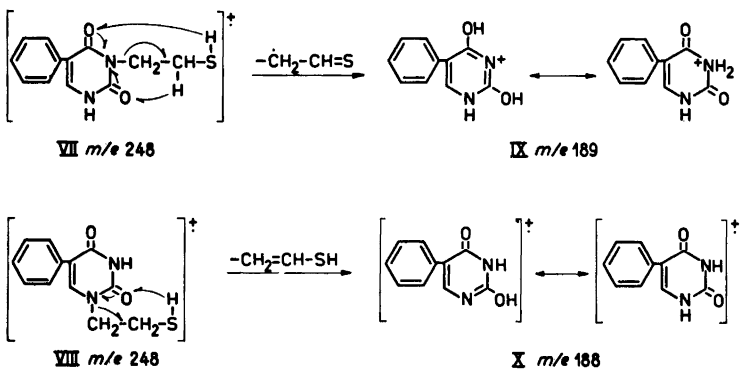


Fig. 2.

The most striking difference in the mass spectra of VII and VIII is the base peak at *m/e* 189 (ion IX) in the former, and the abundant peak at *m/e* 188 (ion radical X) in the latter. Both ions are formed by loss of the alkyl chain with transfer of two, respectively one hydrogen atom (Scheme 2).

Scheme 2

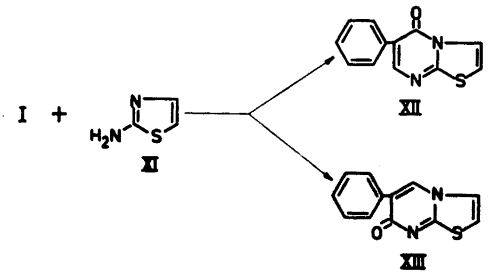


In the first paper in this series [1] we described the mass spectral fragmentation of 1- and 3-alkyluracils and noted, that 1-alkyluracils lose the alkyl chain with transfer of one hydrogen atom while the fragmentation of 3-alkyluracils occurs with double hydrogen rearrangement. Otherwise, the fragmentations of VII and VIII are identical with those previously described for *N*-alkyluracils, except for the 76 mass unit of the phenyl group. The occurrence of the base peak at *m/e* 189, arising from the ion IX, clearly identifies the isomer VII as a 3-alkylated uracil. Hence, the condensation product of I and II in glacial acetic acid (m.p. 181°) is assigned the structure III, and the 233°-isomer obtained in ethanol the structure IV. Further confirmation of this conclusion is found in the fact that the UV maxima of VII undergo

bathochromic shifts in base, a behaviour previously shown to be typical for 3-alkyluracils.[1,5]

In the analogous condensation of ethyl α-formylphenylacetate (I) and 2-aminothiazole (XI) (Scheme 3) in boiling glacial acetic acid, only one, XII, of the two possible isomers was obtained.

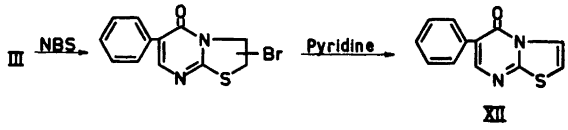
Scheme 3



When the condensation was carried out in ethanol at room temperature the isolated product was identified by NMR as one of the two intermediates XIV or XV. The UV-spectrum indicated the structure of the intermediate to be XIV.

Subsequent treatment of XIV in boiling acetic acid gave the isomer XII obtained directly from I and XI under the same conditions. Attempts to assign the structure by mass spectrographic analysis failed because of resistance of XII to acid hydrolysis. Furthermore, a pronounced instability under basic hydrolytic conditions resulted in destruction of the product. To allow a direct comparison, compound III was converted to its 2,3-dehydro derivative by reaction with *N*-bromosuccinimide followed by dehydrobromination in pyridine.

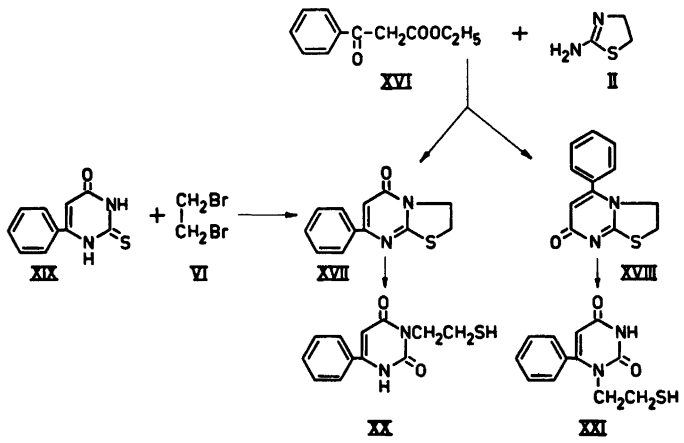
Scheme 4



Although 6-phenyl-5*H*-thiazolo[3,2-a]pyrimidin-5-one (XII) could only be obtained in an impure state, conventional methods such as thin-layer chromatography and UV spectroscopy clearly established the identity of the condensation product with XII. The results demonstrate the different behaviour of 2-amino-2-thiazoline and 2-aminothiazole in the condensation reaction with α-formylphenylacetate in alcohol.

A similar problem of structure assignment was encountered in the reaction of ethyl benzoylacetate (XVI) and 2-amino-2-thiazoline (Scheme 5).

Scheme 5



The only compound isolated (XVII) was identical with the product from the reaction of 6-phenyl-2-thiouracil (XIX) and ethylene bromide. The mass spectrum of XVII provided no basis for a direct structure assignment. The hydrolysis product, however, gave a mass spectrum (Fig. 3) with a base peak at *m/e* 189, *i.e.* the alkyl chain of XX is lost with transfer of two hydrogen atoms. Accordingly, the hydrolysis product XX was assigned to the 3-alkyluracil series, and the condensation product possesses the structure XVII.

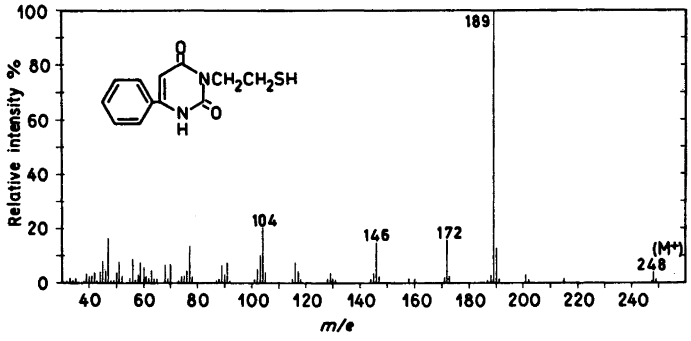


Fig. 3.

The bathochromic shift in base of the UV maxima provided further support for the mass spectral assignment of the hydrolysis product. According to the classification of Allen *et al.*[4] the UV spectrum of XVII in itself indicated that the 2,3-dihydrothiazolopyrimidinone possessed the structure XVIII. Clearly, the UV method is inapplicable to fused pyrimides of the type XVII and XVIII.

EXPERIMENTAL

All mass spectra were obtained with an LKB 9000 mass spectrometer fitted with an all-glass heated inlet system maintained at 130–140°. An ionizing potential of 70 eV was used.

All melting points are uncorrected. Microanalyses were performed by Mr. P. Hansen, The University of Copenhagen, Denmark.

2,3-Dihydro-6-phenyl-5H-thiazolo[3,2-a]pyrimidin-5-one (III). a. To a solution of sodium (6.0 g, 0.26 mole) in ethanol was added 36.0 g (0.26 mole) of 2-amino-2-thiazoline hydrochloride. The precipitate was filtered off and the filtrate evaporated *in vacuo*. The residue was dissolved in 210 ml of glacial acetic acid and ethyl α-formylphenylacetate[6] (55.0 g, 0.286 mole) added. After reflux for 8 h the acetic acid was removed *in vacuo* and the residue recrystallized three times from 2-propanol. Yield 13 g (22 %), m.p. 181°. (Found: C 62.50; H 4.63; N 11.91; S 14.00; Calc. for $C_{12}H_{10}N_2OS$: C 62.58; H 4.38; N 12.17; S 13.92). Principal mass spectral peaks (relative intensity > 5 %): 39 (5); 44 (6); 63 (6); 76 (5); 85 (8); 89 (9); 102 (18); 115 (11); 116 (19); 142 (11); 201 (6); 202 (8); 229 (40); 230 (100); 231 (16); 232 (6). UV (EtOH): λ_{max} 208 nm (ε 14 000), λ_{max} 253 nm (ε 7 800), λ_{max} 314 nm (ε 14 600).

b. To a refluxing suspension of $NaHCO_3$ (1.8 g, 0.021 mole) and ethylene bromide (2.7 ml, 0.031 mole) in 14 ml of 2-propanol was added a solution of 1.0 g (0.0050 mole) of 5-phenyl-2-thiouracil[7] and 0.20 g (0.0050 mole) of NaOH in 35 ml of 60 % aqueous 2-propanol. The mixture was refluxed for 3 h and evaporated to dryness *in vacuo*. The residue was suspended in 25 ml of water and the insoluble compound filtered off and washed with water. Yield: 0.85 g (73 %). The IR- and UV-spectra showed that the product mainly consists of the title compound contaminated with a small amount of the isomer. After two recrystallizations from 2-propanol the m.p. and the spectra were identical with those of the compound described under *a*.

2,3-Dihydro-6-phenyl-7H-thiazolo[3,2-a]pyrimidin-7-one (IV). To an alcoholic solution of 2-amino-2-thiazoline prepared from 21.5 g (0.155 mole) of the hydrochloride was added 29.8 g (0.155 mole) of ethyl α-formylphenylacetate. The mixture was left at room temperature for four days and the precipitate collected. Yield: 29.5 g (83 %), m.p. 159–200°. After two recrystallizations from EtOH–DMF the yield was 18.7 g (52 %), and the m.p. 233°. (Found: C 62.72; H 4.42; N 12.30; S 13.96. Calc. for $C_{12}H_{10}N_2OS$: C 62.58; H 4.38; N 12.17; S 13.92). Principal mass spectral peaks (relative intensity >5 %): 36 (9); 39 (7); 44 (52); 51 (7); 59 (6); 60 (25); 63 (9); 76 (11); 77 (7); 85 (5); 86 (7); 89 (11); 102 (100); 103 (15); 115 (7); 116 (6); 202 (31); 203 (6); 229 (38); 230 (74); 231 (13).

UV (EtOH): λ_{infl} 209 nm (ε 10 900), λ_{max} 231 nm (ε 20 900), λ_{max} 291 nm (ε 11 700).

3-(2-Mercaptoethyl)-5-phenyluracil (VII). a. A suspension of 1.00 g (0.0043 mole) of III in 25 ml of 3 N NaOH was refluxed for 3 h. The solution obtained was acidified with HCl and the precipitate collected. Yield after two recrystallizations from EtOH: 0.58 g (54 %), m.p. 167–168°. (Found: C 58.30; H 4.95; N 11.44; S 12.98; Calc. for $C_{12}H_{12}N_2O_2S$: C 58.04; H 4.87; N 11.28; S 12.91). UV (EtOH): λ_{max} 208 nm (ε 12 200), λ_{max} 238 nm (ε 11 900), λ_{max} 280 nm (ε 8 400). UV (0.1 N NaOH): λ_{max} 219 nm, λ_{max} 250 nm, λ_{max} 301 nm.

b. A suspension of 0.30 g (0.0013 mole) of III in 10 ml of 1 N HCl was refluxed for 20 h. The precipitate was collected and recrystallized from ethanol. Yield: 0.15 g (47 %), m.p. 166–167°.

1-(2-Mercaptoethyl)-5-phenyluracil (VIII). a. A suspension of 1.00 g (0.0043 mole) of IV in 25 ml of 3 N NaOH was refluxed for 2 h. The solution was acidified and the precipitate (1.07 g, 100 %) was collected. After two recrystallizations from EtOH–DMF the yield was 0.75 g (70 %), m.p. 201–203°. (Found: C 58.20; H 4.92; N 11.26; S 12.89.

Calc. for $C_{12}H_{12}N_2O_2S$: C 58.04; H 4.87; N 11.28; S 12.91). UV (EtOH): λ_{max} 208 nm (ε 11 600), λ_{max} 238 nm (ε 11 200), λ_{max} 287 nm (ε 10 800). UV (0.1 N NaOH): λ_{max} 220 nm, λ_{infl} 237 nm, λ_{max} 281 nm.

b. A suspension of 0.30 g (0.0013 mole) of IV in 10 ml of 1 N HCl was refluxed for 20 h. Yield: 0.29 g (90 %), m.p. 202–204°.

6-Phenyl-5H-thiazolo[3,2-a]pyrimidin-5-one (XII). a. A mixture of 25.0 g (0.13 mole) of ethyl α-formylphenylacetate and 13.0 g (0.13 mole) of 2-aminothiazole in 60 ml of glacial acetic acid was refluxed for 6 h. The acetic acid was removed *in vacuo* and the residue recrystallized three times from aqueous ethanol. Yield: 9.1 g (31 %), m.p. 147–148°. (Found: C 62.85; H 3.44; N 12.02; S 14.14; Calc. for $C_{12}H_8N_2OS$: C 63.14; H 3.53; N 12.28; S 14.05). UV (EtOH): λ_{max} 208 nm (ε 13 600), λ_{max} 224 nm (ε 17 000), λ_{max} 271 nm (ε 6 600), λ_{max} 342 nm (ε 16 800).

b. To a suspension of 0.50 g (0.00217 mole) of III and 0.39 g (0.00219 mole) of *N*-bromosuccinimide in 8 ml of CCl_4 was added a few crystals of benzoyl peroxide and the mixture refluxed under UV-light for 2 h. After decantation of the liquid the sticky precipitate was extracted with a further 10 ml portion of CCl_4. After evaporation of CCl_4 the residue was dissolved in 5 ml of benzene and 0.5 ml of pyridine was added and the mixture refluxed for 2 h. Benzene (5 ml) and 3 N HCl (5 ml) were added, the layers were separated, the benzene washed twice with 5 ml of water, and dried over $MgSO_4$. Evaporation of the benzene gave 0.35 g of an oil which slowly crystallized. The UV-spectrum showed it to be a mixture of the starting compound (III) and the title compound (XII). TLC on silicagel (fluorescent) in $CHCl_3$-acetone (9:1) gave a grey spot with R_F=0.32 and a blue spot with R_F=0.38 in UV-light. TLC of III and XII give a grey spot with R_F=0.33 and a blue spot with R_F=0.38, respectively.

c. A solution of 0.30 g (0.0011 mole) of XIV in 3 ml of glacial acetic acid was refluxed for 5 h. The acetic acid was removed *in vacuo* and the residue recrystallized from EtOH. The yield was 0.10 g (40 %); m.p. 147–148°.

N-(2-Carbethoxy-2-phenylethylenyl)-2-imino-4-thiazoline (XIV). A solution of 1.9 g (0.010 mole) of ethyl α-formylphenylacetate and 1.0 g (0.010 mole) of 2-aminothiazole in 5 ml of EtOH was left at room temperature for 7 days. The precipitate was collected and recrystallized from EtOH. Yield 0.55 g (20 %), m.p. 131–132°. (Found: C 61.00; H 5.16; N 10.02; S 11.56. Calc. for $C_{14}H_{14}N_2O_3S$: C 61.29; H 5.14; N 10.21; S 11.69). NMR ($CDCl_3$): 1.25 ppm (triplet) 4.21 ppm (quartet), 6.64 ppm (doublet), 7.08 ppm (doublet), 7.28 ppm (multiplet), 7.83 ppm (broad singlet), 8.04 ppm (singlet). UV (EtOH): λ_{max} 205 nm (ε 14 100), λ_{max} 318 nm (ε 27 600).

2,3-Dihydro-7-phenyl-5H-thiazolo[3,2-a]pyrimidin-5-one (XVII). a. 2-Amino-2-thiazoline prepared from 41.6 g (0.30 mole) of the hydrochloride was dissolved in 240 ml of glacial acetic acid and ethyl benzoylacetate (63.4 g, 0.33 mole) added. After reflux for 5 h the acetic acid was removed *in vacuo* and the residue recrystallized once from EtOH and once from acetonitrile. Yield 11.5 g (17 %), m.p. 164–167°. (Found: C 62.55; H 4.59; N 12.43; S 14.17. Calc. for $C_{12}H_{10}N_2OS$: C 62.58; H 4.38; N 12.17; S 13.92). UV (EtOH): λ_{max} 209 nm (ε 22 500), λ_{max} 255 nm (ε 28 000), λ_{max} 314 nm (ε 5 600).

b. 2-Amino-2-thiazoline prepared from 3.3 g (0.032 mole) of the hydrochloride and ethyl benzoylacetate (6.2 g, 0.032 mole) were dissolved in 10 ml of EtOH and left at room temperature for 7 days. The precipitate was collected and recrystallized form EtOH. Yield: 2.31 g (31 %), m.p. 162°.

c. To a refluxing suspension of 5.5 g (0.065 mole) of $NaHCO_3$ and 8.0 ml (0.093 mole) of ethylene bromide in 40 ml of 2-propanol was added a solution of 3.0 g (0.015 mole) of 6-phenyl-2-thiouracil [8] and 0.60 g (0.015 mole) of NaOH in 100 ml of 60 % 2-propanol. The mixture was refluxed for 3 h., filtered, and evaporated to dryness *in vacuo*. The residue was suspended in water and the insoluble compound (2.6 g, 75 %), filtered off. Recrystallization from EtOH yielded 2.4 g (69 %), m.p. 163–165°.

3-(2-Mercaptoethyl)-6-phenyluracil (XX). The compound was prepared by hydrolysis of XVII in acid or base as described above for VII, and recrystallized from 2-propanol. Base hydrolysis: Yield 53 %, m.p. 224–226°. Acid hydrolysis: Yield 78 %, m.p. 225°. (Found: C 58.14; H 4.95; N 11.33; S 12.99. Calc. for $C_{12}H_{12}N_2O_2S$: C 58.04; H 4.87; N 11.28; S 12.91). UV (EtOH): λ_{max} 209 nm (ε 13 500), λ_{infl} 222 nm (ε 12 700), λ_{max} 286 nm (ε 11 900). UV (0.1 N NaOH): λ_{infl} 225 nm, λ_{max} 235 nm, λ_{max} 309 nm.

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