SYNTHESIS OF 3-(6-AZAURACIL-5-YL)ANTHRANILIC ACID AND ITS APPLICATION TO THE PREPARATION OF OTHER 1,2,4-TRIAZINE DERIVATIVES*

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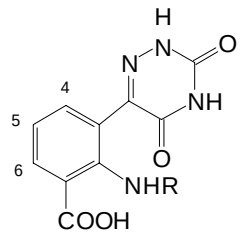
The title compound was synthesized by alkaline recyclization of isatin-7-carboxylic acid semicarbazone and used for the preparation of 3-oxo-2,3-dihydro-5*H*-1,2,4-triazino[5,6-*b*]indol-6-carboxylic acid (**8**) and 3-oxo-2,3,4,6-tetrahydro-1,2,4-triazino[5,6-*c*]cinnoline-7-carboxylic acid (**9**).
Key word: 1,2,4-Triazines.

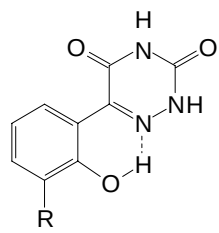
Current interest in polynuclear heterocyclic compounds is related, among other things, with efforts to discover new substances capable of intercalation into the double-helix of DNA, and/or with the seeking for additional compounds inhibiting topoisomerases[2]. Within our study of cyclization reactions leading to unfused as well as fused 1,2,4-triazines we concerned ourselves with the synthesis of 3-(6-azauracil-5-yl)anthranilic acid (**1**), which served as the starting substance in the synthesis of some other 1,2,4-triazine derivatives which are of interest with respect to the capabilities mentioned. We were able to obtain the acid in a good yield by alkaline recyclization of isatin-7-carboxylic acid semicarbazone. Additional conversions of this compound were related with the reactivity of the amino group, located midway between the carboxy group and the rather bulky 6-azauracil group. The cyclization to 3-oxo-2,3-dihydro-1,2-4-triazino[5,6-*b*]-indole-6-carboxylic acid (**8**), achieved by boiling in acetic acid, was straightforward. No problems were encountered either in reactions where the effect of steric hindrance could actually be expected. This concerns benzoylation resulting in the benzoyl derivative **2**, as well as diazotization. Coupling reactions of solutions of this diazonium salt with malonic dinitrile and ethyl cyanoacetate giving the hydrazones **3** and **4**, respectively, proceeded smoothly as well. Coupling with an alkali sulfite and reduction of the formed diazosulfonate by the excess sulfite gave hydrazine sulfonate, whose acid

* Part III in the series Cyclocondensation Reactions of Heterocyclic Carbonyl Compounds; Part II: see ref.[1].

hydrolysis resulted in 2-hydrazino-3-(5-azauracil-5-yl)benzoic acid (**5**). This compound was subject to cyclization by boiling in an acid solution. The direction of this cyclization was an interesting problem, because both the closing of the indazole cycle, as is the case with 2-hydrazinobenzoic acid[3], and the closing of the cinnoline cycle, as is the case with 5-(2-hydrazinophenyl)-6-azauracil[4], were feasible. We found that the latter pathway was preferred: 3-oxo-2,3,4,6-tetrahydro-1,2,4-triazino[5,6-*c*]cinnoline-7-carboxylic acid (**9**) emerged, as evidenced by the ^{1}H NMR spectrum exhibiting only three NH protons (at 11.87, 12.24 and 13.66 ppm) as singlets. The compound **9** was also obtained by boiling the hydrazones **3** and **4** in strongly acid solutions, during which the substances were subject to hydrolytic cleavage to give the hydrazine derivative **5**, which underwent cyclization.

The problem of the possible cyclization of the benzoyl derivative **2** was also examined. As expected, cyclization to the compound **8** or its 6-benzoyl derivative did not take place even during a long-term boil in acetic acid. Boiled in hydrochloric acid, however, the substance transformed readily into the derivative **8** while splitting off the benzoyl group.





1, R = H

2, R = COPh

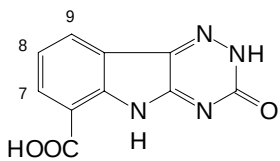
3, R = N=C(CN)$_2$

4, R = N=C(CN)COOC$_2$H$_5$

5, R = NH$_2$

6, R = H

7, R = COOH



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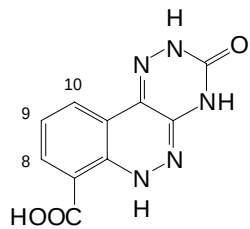

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Heated in an aqueous solution, the diazotized acid **1** underwent facile nucleophilic substitution giving 3-(6-azauracil-5-yl)salicylic acid (**7**). For a comparison, 5-(2-hydroxyphenyl)-6-azauracil (**6**) was prepared analogously from diazotized 5-(2-aminophenyl)-6-azauracil. For the compounds **1**, **6**, and **7**, their conformation and existence or nonexistence of intramolecular hydrogen bonds in them were of interest. Their low solubility only permitted the infrared spectra to be measured in the solid state. The spectrum of the acid **1** exhibited a well-developed carbonyl stretching band at 1 655 cm^{-1} belonging to the carboxy group; this rather low wavenumber, as well as the occurrence of broad bands at 2 562 and 2 643 cm^{-1} due to the carboxy OH group stretching vibrations, gives evidence of a mutual bonding of the carboxylic groups in dimers. The NH_2 stretching band positions, 3 484 and 3 453 cm^{-1}, do not indicate association of the carboxylic and/or amino groups or ionization of the latter. These conclusions are consistent with those arrived at during the study of anthranilic acid[5].

The acid **7** is monomeric in the solid state. This is evidenced by the high stretching vibration wavenumber of the carboxylic carbonyl group, lying at 1 753 cm^{-1}. This band vanishes when the acid is converted to its salt, and intense bands due to the symmetric and antisymmetric stretching vibrations of the carboxylate anion appear at 1 409 and 1 582 cm^{-1}. The phenolic hydroxy group, which is bonded by a hydrogen bond to the nitrogen atom in position 6 of the 6-azauracil ring, gives rise to a band at 3 254 cm^{-1}. Comparison with the compound **6** indicates that the presence of the carboxy group induces shifts of the bands due to the stretching vibrations of the bonded hydroxy group and of the carbonyl group to 3 268 and 1 710 cm^{-1}, respectively.

EXPERIMENTAL

The melting points were determined on a Boetius stage and are not corrected. The infrared spectra were measured in KBr disks and scanned on an ATI Unicam Genesis FTIR instrument. The NMR spectra were measured in solutions in hexadeuteriodimethyl sulfoxide on a Bruker AMX-360 spectrometer (360 MHz); the chemical shifts reported are in ppm. Elemental analyses were performed by using an EA 1108 Elemental Analyser (Fison Instrument).

2-Amino-3-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)benzoic Acid (**1**)

Isatin-7-carboxylic acid semicarbazone[6] (1.30 g, 5.24 mmol) was refluxed in 1 M NaOH (50 ml) for 4 h, after which the system was filtered and made acidic to pH 5 using acetic acid. The next day the precipitate formed was separated, rinsed with water, and dried at 110 °C. Yield 1.16 g (89%), m.p. 285 °C (ethanol). For $C_{10}H_8N_4O_4$ (248.2) calculated: 48.39% C, 3.25% H, 22.57% N; found: 48.54% C, 3.23% H, 22.09% N. IR spectrum: 3 484, 3 453 (NH_2), 3 364 (NH), 2 643, 2 562 (OH), 1 747 (C=O), 1 655 (C=O carbonyl). ^{1}H NMR spectrum: 6.61 t, 1 H, J = 7.7 (H-5); 7.34 d, 1 H, J = 7.3 (H-4); 7.85 d, 1 H, J = 7.4 (H-6); 12.02 s, 1 H (NH); 12.41 s, 1 H (NH).

2-Benzoylamino-3-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)benzoic Acid (**2**)

The compound **1** (73.2 mg, 0.29 mmol) was dissolved in pyridine (3 ml), and benzoyl chloride (40.8 mg, 0.29 mmol) was added. The next day the mixture was diluted with water (50 ml). The precipitate

formed was filtered out, rinsed with water, and dried. Yield 86.8 mg (85%), m.p. over 320 °C (ethanol). For $C_{17}H_{12}N_4O_5$ (352.3) calculated: 57.96% C, 3.43% H, 15.90% N; found: 58.01% C, 3.33% H, 15.60% N. IR spectrum: 1 742, 1 712, 1 685 (C=O), 1 624 (C=C), 770 (C–H arom.).

2-Dicyanomethylenehydrazino-3-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)benzoic Acid (**3**)

The compound **1** (103.1 mg, 0.42 mmol) was dissolved in 1 M NaOH (5 ml), and $NaNO_2$ (36.2 mg, 0.52 mmol) was added. The solution was cooled to 0–5 °C and added slowly to a mixture of water (5 ml) and concentrated HCl (1 ml) cooled to the same temperature. After being allowed to stand in an ice bath for 2 h, the system was added to a cold mixture of malonic dinitrile (51.4 mg, 0.78 mmol), sodium acetate (1 g, 12.19 mmol), and NaOH (400 mg, 10 mmol) in water (15 ml). The mixture was left in a refrigerator overnight and made acidic with HCl to pH 2, and in 3 days the precipitate formed was filtered out, rinsed with water, and dried at 120 °C. Yield 129.6 mg (95%), m.p. 247 °C (dec., ethanol). For $C_{13}H_7N_7O_4$ (325.2) calculated: 48.01% C, 2.17% H, 30.15% N; found: 47.85% C, 2.21% H, 29.91% N. IR spectrum: 2 240 (CN), 1 725 (C=O), 1 686 (C=O), 1 609 (C=N), 768 (C–H).

2-Cyano(ethoxycarbonyl)methylenehydrazino-3-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)benzoic Acid (**4**)

The compound **1** (101.1 mg, 0.41 mmol) was dissolved in 1 M NaOH (5 ml), and $NaNO_2$ (36.0 mg, 0.52 mmol) was added. The solution was cooled to 0–5 °C and added slowly to a mixture of water (5 ml) and concentrated HCl (1 ml) at the same temperature. The solution was allowed to stand in an ice bath for 2 h and added to a cold mixture of ethyl cyanoacetate (60 mg, 0.53 mmol), sodium acetate (2 g, 24.4 mmol), water (15 ml), and ethanol (2.5 ml). The system was left in a refrigerator for 4 days. The precipitate formed was filtered out, rinsed with water, and dried. Yield 143.5 mg (94%), m.p. 247 °C (dec., ethanol). For $C_{15}H_{12}N_6O_6$ (372.3) calculated: 48.39% C, 3.25% H, 22.57% N; found: 48.02% C, 3.11% H, 22.33% N. IR spectrum: 2 990 (C–H ethyl), 2 219 (CN), 1 726 (C=O), 1 690 (C=O carboxyl).

5-(*o*-Hydroxyphenyl)-6-azauracil (**6**)

5-(*o*-Aminophenyl)-6-azauracil[7] (500 mg, 2.45 mmol) was dissolved in 2% NaOH (5 ml), and $NaNO_2$ (175 mg, 2.54 mmol) was added. This mixture was added cautiously to a 10% solution of H_2SO_4 so that the temperature did not exceed 0–5 °C, at which the solution was stirred for an hour. The system was left in a refrigerator overnight, and the next day water was added (15 ml) and the mixture was allowed to boil for 1 h. After cooling down, the precipitate was filtered out, rinsed with water, and dried. Yield 457.2 mg (91%), m.p. 299 °C (dec., water). For $C_9H_7N_3O_3$ (205.2) calculated: 52.71% C, 3.44% H, 20.49% N; found: 52.74% C, 3.23% H, 20.22% N. IR spectrum: 3 268 (OH), 3 019 (C–H arom.), 1 710 (OH), 1 699 (C=O), 762 (C–H arom.).

2-Hydroxy-3-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)benzoic Acid (**7**)

The compound **1** (300 mg, 1.21 mmol) was dissolved in 2% NaOH (5 ml), and $NaNO_2$ (100 mg, 1.45 mmol) was added. This mixture was added cautiously to a 10% solution of H_2SO_4 (5 ml) so that the temperature did not exceed 0–5 °C, at which the solution was stirred for an hour. The system was left in a refrigerator overnight, and the next day water was added (10 ml) and the mixture was allowed to boil for 1 h. After cooling down, the precipitate was filtered out, rinsed with water, and dried. Yield 182 mg (63%), m.p. 296–298 °C (water). For $C_9H_7N_3O_5$ (237.2) calculated: 45.57% C, 2.97% H,

17.72% N; found: 45.32% C, 2.94% H, 17.44% N. IR spectrum: 3 252 (OH), 3 023 (C–H arom.), 1 753 (C=O carboxyl), 1 692 (C=O). ^{1}H NMR spectrum: 7.04 t, 1 H, *J* = 7.7 (H-5); 7.6 d, 1 H, *J* = 7.5 (H-4); 7.95, 1 H, *J* = 7.5 (H-6); 12.16 s, 1 H (NH); 12.53 s, 1 H (NH); 11.68 brs, 1 H (OH).

3-Oxo-2,3-dihydro-5*H*-1,2,4-triazino[5,6-*b*]indole-6-carboxylic Acid (**8**)

Method A. A solution of the compound **1** (1.00 g, 4.03 mmol) in glacial acetic acid (80 ml) was refluxed for 40 min. After cooling down, the precipitate formed was filtered out, rinsed with water, and dried. Yield 720 mg (78%), m.p. over 320 °C (acetic acid). For $C_{10}H_6N_4O_3$ (230.2) calculated: 52.18% C, 2.63% H, 24.34% N; found: 51.89% C, 2.59% H, 24.01% N. IR spectrum: 1 692 (C=O), 1 661 (C=O), 1 610 (C=N). ^{1}H NMR spectrum: 7.37 t, 1 H, *J* = 7.6 (H-8); 8.04 d, 1 H, *J* = 7.4 (H-9); 8.14 d, 1 H, *J* = 7.5 (H-7); 11.55 brs, 1 H (NH); 13.24 s, 1 H (NH).

Method B. A mixture of the compound **2** (200 mg, 0.57 mmol) and 20% HCl (20 ml) was refluxed for 3 h. After cooling down, the precipitate formed was filtered out, rinsed with water, and dried. Yield 122 mg (93%). The characteristics of the compound were as given sub *A*.

3-Oxo-2,3,4,6-tetrahydro-1,2,4-triazino[5,6-*c*]cinnoline-7-carboxylic Acid (**9**)

Method A. The compound **1** (400 mg, 1.61 mmol) was dissolved in a 2.5% solution of ammonia (22 ml), and $NaNO_2$ (130 mg, 1.9 mmol) was added. The mixture was added dropwise to a 10% HCl solution (15 ml) so that the temperature did not exceed 0–5 °C. The mixture was stirred till complete dissolution and allowed to stand at this temperature for 15 min. Subsequently, a solution of $Na_2SO_3 . 7 H_2O$ (3.5 g, 13.9 mmol) in water (10 ml) was added, and the mixture was allowed to stand in a refrigerator overnight. The next day the solution was heated at 80 °C for 1 h, made acidic with concentrated HCl (10 ml), and refluxed for 10 h. The precipitate which formed in several hours of standing was filtered out, rinsed with water, and dried. Yield 270 mg (68%), m.p. over 320 °C (acetic acid). For $C_{10}H_7N_5O_3$ (245.2) calculated: 48.98% C, 2.88% H, 28.56% N; found: 48.57% C, 2.91% H, 28.11% N. IR spectrum: 1 764 (C=O), 1 710 (C=O), 1 674 (C=N). ^{1}H NMR spectrum: 7.42 t, 1 H, *J* = 7.3 (H-9); 8.10 d, 1 H, *J* = 7.4 (H-10); 8.43 d, 1 H, *J* = 7.4 (H-8); 11.87 s, 1 H (NH); 12.24 s, 1 H (NH); 13.66 s, 1 H (NH).

Method B. The compound **3** (80 mg, 0.25 mmol) was boiled in a mixture of acetic and hydrochloric acids (5 ml each) for 11 h. The precipitate formed was filtered out, rinsed with water, and dried. Yield 40.3 mg (66%), characteristics as given sub *A*.

Method C. Compound **4** (110 mg, 0.3 mmol) was treated as sub *B*. Yield 50.1 mg (69%), characteristics as sub *A*.

REFERENCES

1. Hlavac J., Slouka J.: Acta Univ. Palacki. Olomuc., Fac. Rerum Nat. *117*, Chem. XXXIII, 65 (1994).
2. Isaacs R. J., Davies S. L., Wells N. J., Harris A. L.: Anticancer Drugs *6*, 195 (1995).
3. Stephenson E. F. M.: Org. Synth., Coll. Vol. *III*, 475 (1955).
4. Slouka J.: Collect. Czech. Chem. Commun. *44*, 2438 (1979).
5. Kellie A. E., O'Sullivan D. G., Sadler P. W.: J. Org. Chem. *22*, 29 (1957).
6. Rupe H., Guggenbuhl G. A.: Helv. Chim. Acta *10*, 926 (1927).
7. Ioffe I. S., Tomchin A. B., Rusakov E. A.: Zh. Obshch. Khim. *39*, 2345 (1969).