46. *Synthesis of Antiviral Agents. Part I. Heterocyclic Compounds Related to Isatin 3-Thiosemicarbazone.*

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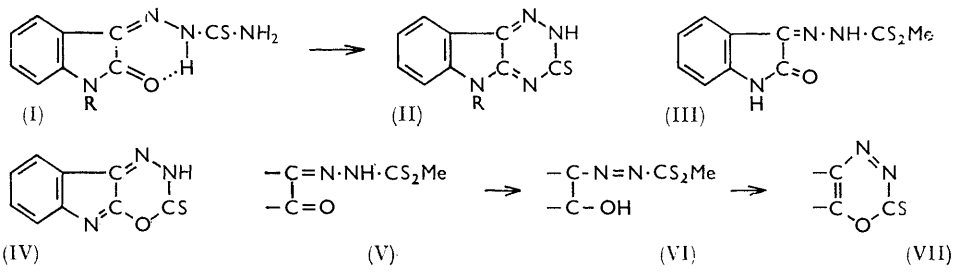
Diphenyl derivatives of dihydrothio-1,2,4-triazine and 5-thio-2-triazoline have been synthesised and found to have no antiviral activity; indole-5-thioamide is also inactive. Some ring-substituted *N*-alkylisatin 3-thiosemicarbazones showed high activity.

1-ETHYLISATIN 3-THIOSEMICARBAZONE (I; R = Et), the most potent antiviral agent yet discovered, is under clinical trial.[1,2] As very few heterocyclic thiosemicarbazones possess detectable antiviral activity, the possibility arises that 1-alkylisatin 3-thiosemicarbazones owe their high activity to conversion *in vivo* into tricyclic dihydrothioindolotriazines (II). Both the α-carbonyl and the terminal amino-group (*i.e.*, the groups necessary for cyclization) are essential for the retention of antiviral activity.[1] Although the 2-carbonyl group is part of an amide grouping, there are cases in which both carbonyl groups of isatin take part in a reaction. Thus 1-methylisatin with two mols. phenylmagnesium bromide gives a mixture of indoxyl and oxindole derivatives;[3] 1-acetylisatin gives a dioxime[4] and bisphenylhydrazone;[5] isatin gives a dioxime,[6] dianilide,[7] and a quinoxaline derivative.[8]

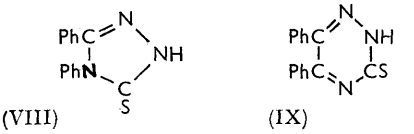
Attempts to prepare a compound (II; R = Me) by heating 1-methylisatin 3-thiosemicarbazone above its melting point or by reaction with strong acids failed. Heating 1-ethylisatin 3-thiosemicarbazone under reflux in butanol for 14 days produced only 1-ethylisatin 3-4′-butylthiosemicarbazone. No antiviral activity was detected in any of

[1] Bauer and Sadler, *Brit. J. Pharmacol.*, 1960, **15**, 101
[2] Bauer and Sadler, *Lancet*, 1960, **1**, 1110.
[3] Witkop and Ek, *J. Amer. Chem. Soc.*, 1951, **73**, 5664.
[4] Schunk and Marchlewski, *Ber.*, 1895, **28**, 543.
[5] Panaotovik, *J. prakt. Chem.*, 1886, **33**, 58.
[6] Reissert and Hessert, *Ber.*, 1924, **57**, 964.
[7] Reissert and Hoppmann, *Ber.*, 1924, **57**, 972.
[8] Buraczewski and Marchlewski, *Ber.*, 1901, **34**, 4010; Korczynski and Marchlewski, *Ber.*, 1902, **35**, 4334.

the decomposition products formed in these reactions. Cyclisation of isatin 3-*N'*-methylthiothiocarbonylhydrazone (III) to the oxadiazine (IV) has been reported by Guha and Guha.[9] The reaction is a general one; for example, phenanthraquinone and acenaphthaquinone form the thioesters (V), which, in the azo-form (VI) lose methanethiol, giving the 1,3,4-oxadiazines (VII). Repetition of the preparation of compound (IV) yielded a



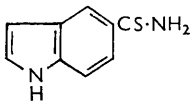
compound of melting point similar to that reported,[9] but analysis indicated structure (III), which was confirmed when the infrared spectra disclosed the presence of two imino-groups and a carbonyl group. Similarly 1-methylisatin produced only a derivative (V), and none of the oxadiazine (VII). However, heating the hydrazone (III) in acetic anhydride yielded its acetyl derivative and this had a melting point similar to that reported for the acetyl derivative of the oxadiazine (IV);[9] the benzoyl derivative of compound (IV) was obtained in small yield from the hydrazone (III) by the Schotten–Baumann process. This is analogous to the action of excess of benzoyl chloride on 1-benzoyl-4-phenylthiosemicarbazide which cyclises with loss of water to form 3,4-diphenyl-5-thio-2-triazoline (VIII). The corresponding triazine (IX) is formed more readily, by heating an ethanolic solution of benzil monothiosemicarbazone under reflux. From this it follows that the 2-carbonyl group of isatin is not sufficiently reactive to permit cyclisation of its monothiosemicarbazone and methylthiothiocarbonylhydrazone under mild conditions, which is a characteristic of some other α-diketones. Further evidence against the postulated conversion of 1-alkylisatin 3-thiosemicarbazones *in vivo* into indolotriazines is that neither the thiotriazoline (VIII) nor the dihydrothiotriazine (IX) possesses antiviral activity. This, coupled with the lack of direct evidence for the formation of (II) from (I), render this mechanism unlikely.



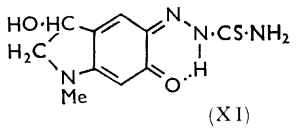
As the formation of stable tricyclic compounds from isatin 3-thiosemicarbazones could not be demonstrated, a study was made of the configuration of their hydrogen-bonded forms. Infrared spectra indicate the presence of both inter- and intra-molecular bonding, the latter being more marked in the 1-alkyl derivatives (I). Similar resonance stabilising of six-membered hydrogen-bonded rings has been demonstrated for isatin 3-oximes.[10] A similar hydrogen-bonded structure is indicated for isatin 2-thiosemicarbazone, but as this has no antiviral activity, the possibility arises that the steric relation of the thioamide group to the ring-nitrogen atom is important. 5-Thiocarbamoylindole (X) has the thioamide group in the required position but has no antiviral activity. Adrenochrome monothiosemicarbazone (XI) is also inactive.

[9] Guha and Guha, *J. Indian Chem. Soc.*, 1927, **4**, 239.
[10] O'Sullivan and Sadler, *J. Org. Chem.*, 1957, **22**, 283.

In view of the high antiviral activity of compounds (I), some derivatives having a substituent also in the benzene ring have been prepared. 3-Thiosemicarbazones of 5-fluoro-1-methyl- and 1-ethyl-5-fluoro-isatin retained the high activity, and the compound having



(X)



(XI)

a 1,7-trimethylene bridge had activity approximately the mean of the values for the 1- and the 7-ethyl compound.

Experimental

Spectra were determined by using a Perkin-Elmer 21 double-beam recording spectrometer with a rock-salt prism.

Test of Antiviral Activity.—The IHD strain of neurotropic vaccinia virus adapted to intracerebral passage in mice was used. The mean reciprocal survival time of mice infected intracerebrally with neurovaccinia virus has a linear regression upon the logarithm of the dilution of virus used for infection; an active compound causes a significant reduction of the mean reciprocal survival time over a wide range of virus doses.[11] Compounds which gave no significant reduction of the mean reciprocal survival time at a dose of 125 mg./kg. in mice infected intracerebrally with about 1000 LD 50 of neurovaccinia virus were considered to be inactive.

1-*Ethylisatin* 3-4′-*Butylthiosemicarbazone.*—1-Ethylisatin 3-thiosemicarbazone (2 g.) was refluxed in butanol (125 c.c.) for 14 days. When the solution was concentrated to 50 c.c. and chilled, 0·1 g. of unchanged thiosemicarbazone, m. p. 204°, was recovered. Further concentration produced crystals which on recrystallisation from ethanol afforded the red 4′-*butyl derivative*, m. p. 83° (Found: C, 59·3; H, 6·2; S, 10·2. $C_{15}H_{20}N_4OS$ requires C, 59·2; H, 6·6; S, 10·5%).

Isatin 3-N′-*Methylthiothiocarbonylhydrazone* (III).—Isatin (1·5 g.) and methylthiothiocarbonylhydrazine (1·3 g.) were heated in acetic acid under reflux; a deep yellow precipitate of the *hydrazone* was obtained which recrystallised from acetone as pale orange needles, m. p. 227° (Found: C, 47·9; H, 3·5; S, 25·5. $C_{10}H_9N_3OS_2$ requires C, 47·8; H, 3·6; S, 25·5%). When this was heated under reflux in acetic anhydride for 20 min., and the product was recrystallised from ethanol, the *acetyl derivative* formed white needles, m. p. 165° (Found: S, 22·2. $C_{12}H_{11}N_3O_2S_2$ requires S, 21·9%). The acetyl derivative of the oxatrazine (IV) (calc. for $C_{11}H_7N_3O_2S$: S, 13·1%) is reported to have m. p. 162°. Schotten–Baumann benzoylation of the hydrazone (III) gave the benzoyloxathiazine as white plates, m. p. 227° (lit., m. p. 228—229°).

1-*Methylisatin* 3-*methylthiothiocarbonyl hydrazone* was obtained similarly as orange needles, m. p. 171° (Found: C, 49·7; H, 4·0; S, 23·5. $C_{11}H_{11}N_3OS_2$ requires C, 49·8; H, 4·2; S, 24·1%).

Adrenochrome Monothiosemicarbazone (XI).—Adrenaline (5 g.) in chilled water (100 c.c.) containing 20N-formic acid (2 c.c.) was added with rapid stirring to silver oxide (20 g.) in chilled water (40 c.c.). The mixture was stirred vigorously for 2 min. Ice (50 g.) was added and the mixture filtered into a solution of thiosemicarbazide (3·7 g.) in the minimum quantity of warm water.[12] The flocculent red *thiosemicarbazone* was collected, washed with water, and recrystallised from ethanol (1 l.), giving red needles, m. p. 220° (Found: C, 47·5; H, 4·6. $C_{10}H_{12}N_4O_2S$ requires C, 47·6; H, 4·8%).

2,3-*Dihydro*-5,6-*diphenyl*-3-*thio*-1,2,4-*triazine* (IX).—Benzil (21·1 g.) and thiosemicarbazide (9·1 g.) were heated under reflux in ethanol (200 c.c.) for 24 hr. The orange precipitate was removed from the cooled mixture, suspended in water (200 c.c.) at 80° for 20 min., collected again, and washed with ether, leaving the *triazine derivative* (13·7 g.) as orange needles which, recrystallised from butanol, had m. p. 226° (Found: C, 67·8; H, 4·1; N, 16·2; S, 12·1.

[11] Bauer, *Brit. J. Exp. Path.*, 1958, **39**, 480.
[12] Fleischhaker and Barsel, U.S.P. 2,712,024/1955.

$C_{15}H_{11}N_3S$ requires C, 67·9; H, 4·2; N, 15·9; S, 12·1%). Chilling the ethanolic filtrate produced benzil monothiosemicarbazone as white needles (1·6 g.), m. p. 188° unchanged on crystallization from ethanol (Found: C, 63·8; H, 4·8. Calc. for $C_{15}H_{13}N_3OS$: C, 63·5; H, 4·6%). Concentration of the final mother-liquors produced a mixture (7·5 g.) of the two products from which the thiosemicarbazone was removed by continuous extraction with ether; neither fractional crystallisation from ethanol nor the action of sodium carbonate solution effected separation.[13] Heating the thiosemicarbazone at 110° at 0·05 mm. for 3 hr. converted it quantitatively into the triazine.

3,4-*Diphenyl*-5-*thio*-2-*triazoline* (VIII).—Benzhydrazide (4·5 g.) was added to phenyl isothiocyanate (4·5 g.) in ethanol (20 c.c.) and left overnight, giving 1-benzoyl-4-phenylthiosemicarbazide (5·3 g.) as needles, m. p. 164°, which was then heated with a large excess of benzoyl chloride for 3 min. on a boiling-water bath. Ether was added to the chilled mixture, and the precipitate collected and washed with ether. Crystallisation from ethanol gave needles of the triazoline, m. p. 284° (lit.,[14] m. p. 287°).

1,2,5,6-*Tetrahydro*-1,2-*dioxo*-4H-*pyrrolo*[3,2,1-ij]*quinoline* (1,7-*Trimethyleneisatin*) 3-*Thiosemicarbazone*.—Equimolar quantities of the isatin [15] and thiosemicarbazide were refluxed in aqueous ethanol. The *product* was collected, washed with hot water, and crystallised from ethanol, forming orange plates, m. p. 236° (Found: C, 55·4; H, 4·4; S, 12·2. $C_{12}H_{12}N_4OS$ requires C, 55·4; H, 4·6; S, 12·3%).

5-*Fluoro*-1-*methylisatin* 3-*Thiosemicarbazone*.—Equimolar quantities of 1-sodio-5-fluoro isatin [16] and methyl iodide were refluxed in ethanol for 48 hr. The mixture was filtered and extracted with benzene. Acidification with concentrated hydrochloric acid of a 2N-sodium hydroxide extract of the benzene solution gave 5-fluoro-1-methylisatin as pale red needles, m. p. 151°. The *thiosemicarbazone*, crystallised from ethanol, had m. p. 260° (Found: C, 47·7; H, 3·6; S, 12·4. $C_{10}H_9FN_4OS$ requires C, 47·6; H, 3·6; S, 12·7%).

1-*Ethyl*-5-*fluoroisatin* 3-*thiosemicarbazone* was prepared similarly. 1-Ethyl-5-fluoroisatin formed red needles, m. p. 131°, and its *thiosemicarbazone* orange needles, m. p. 228° (Found: C, 49·5; H, 4·0; S, 12·2. $C_{11}H_{11}FN_4OS$ requires C, 49·6; H, 4·1; S, 12·0%).

7-*Ethylisatin* 3-*thiosemicarbazone* was obtained as orange plates, m. p. 257° (Found: C, 53·4; H, 5·0; S, 12·9. $C_{11}H_{12}N_4OS$ requires C, 53·2; H, 4·8; S, 12·8%).

5-*Thiocarbamoylindole*.—A solution of 5-cyanoindole (0·1 mole, obtained by the action of cuprous cyanide on 5-bromoindole [17] in quinoline) in 30% (w/v) ammoniacal ethanol (20 c.c.) was saturated with hydrogen sulphide and left for 2 days. The ethanol was removed under reduced pressure and the residual *amide* crystallised several times from hot water, giving white needles, m. p. 162° (Found: C, 61·3; H, 4·4; N, 15·7; S, 18·4. $C_9H_8N_2S$ requires C, 61·4; H, 4·6; N, 15·9; S, 18·2%).

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[13] Thiele and Stange, *Annalen*, 1894, **283**, 1.
[14] Marckwald and Bott, *Ber.*, 1896, **29**, 2914.
[15] Martinet, *Ann. Chim.* (*France*), 1919, **11**, 85.
[16] Holt and Sadler, *Proc. Roy. Soc.*, 1958, *B*, **148**, 481.
[17] Snyder, Hansch, Katz, Parmeter, and Spaeth, *J. Amer. Chem. Soc.*, 1948, **70**, 219.