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SYNTHESIS OF POTENTIAL ANTICANCER AGENTS—I

SYNTHESIS OF SUBSTITUTED THIOPHENES

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Abstract—In view of the anticancer activity of thiophene-2,5-dicarboxylic acid, a series of derivatives of this acid were prepared. Starting from 2,5-dichloromethylthiophene, thiophene-2,5-dialdehyde, thiophene-2,5-dimethylenyl-thiouronium dichloride, 2,5-dimercaptomethylthiophene were prepared. 3,4-Dihydroxylthiophene, a thiophene isoster of catechol, was prepared by decarboxylation of 2,5-dicarboxy-3,4-dihydroxythiophene. Of the compounds reported, the above dithiouronium salt proved highly active against Yoshida sarcoma in rats.

THE synthesis and anticancer activity of thiophene 2,5-dicarboxylic acid[1] (I) and 2,5-dicarbethoxy-3,4-dihydroxythiophene[2] (VI) has been reported and the anticancer activity attributed to their likely interference in the hexose-monophosphate (HMP) pathway. As an extension of this work, the synthesis of thiophene, tetrahydrothiophene, furan, tetrahydrofuran, thiapyran and thia-aromatic compounds has been undertaken. The present paper records the synthesis of some substituted thiophenes.

Thiophene-2,5-dicarboxylic acid (I) was rapidly excreted and thus had to be repeatedly administered in order to maintain its adequate level in the body.[1] With the view to increase its retention *in vivo*, the acid (I) was converted into its dihydrazide (III) and 2,5-dicarbethoxylthiophene (II). The dihydrazide was sparingly soluble in water.

Another compound of interest was 2,5-dicarbethoxy-3,4-dihydroxythiophene (hereafter referred to as Dicetol; VI). The compound may be regarded as a close analog of gluconic acid and its oxidation products which are key intermediates in the HMP pathway. Compound VI was prepared in improved yield (90%) by a modification of the synthesis by Overberger and Joginderlal.[3] Dicetol VI, being insoluble in water and aqueous sodium bicarbonate, was converted into the disodium salt (VII; Disodio-Dicetol) by interaction with a calculated amount of sodium ethoxide in ethanol.

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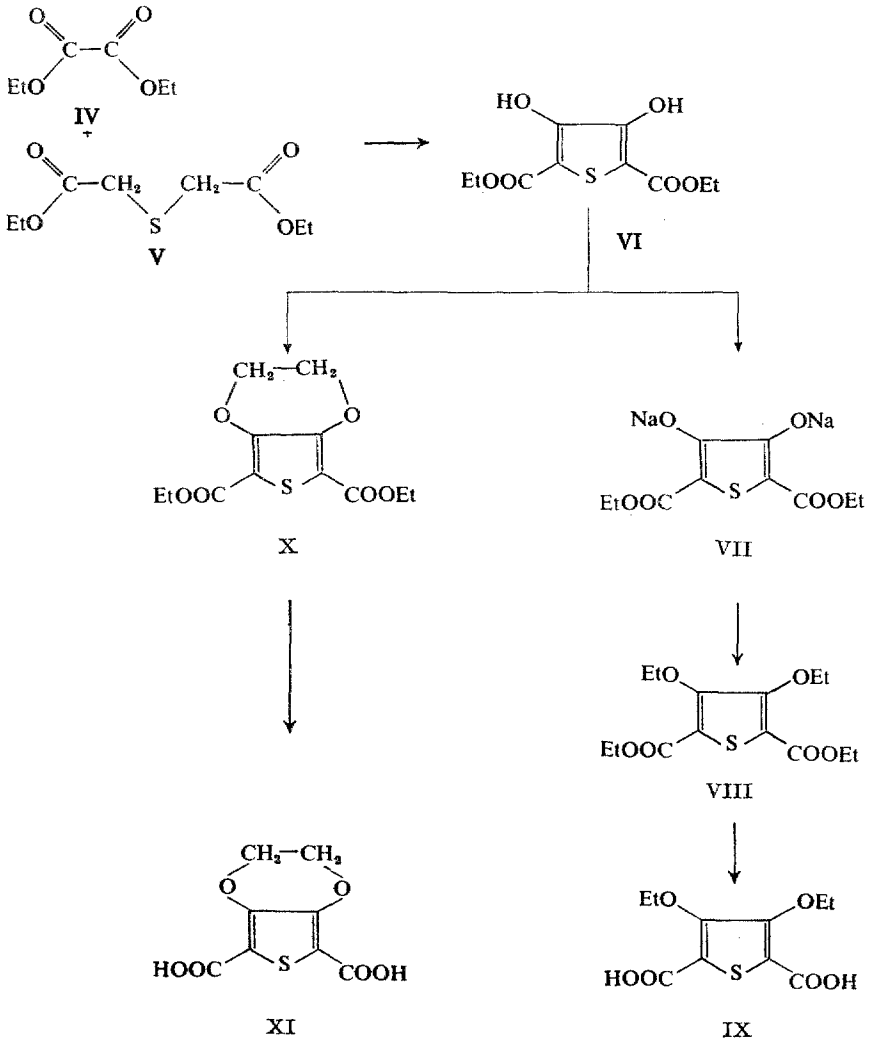
[1] M. B. Sahasrabudhe, M. K. Nerurkar, M. V. Nerurkar, B. D. Tilak and M. D. Bhavasar, *Brit. J. Cancer* **14**, 547 (1960).

[2] M. B. Sahasrabudhe, M. V. Nerurkar, L. B. Kotnis, B. D. Tilak and M. D. Bhavasar, *Nature, Lond.* **184**, 202 (1959).

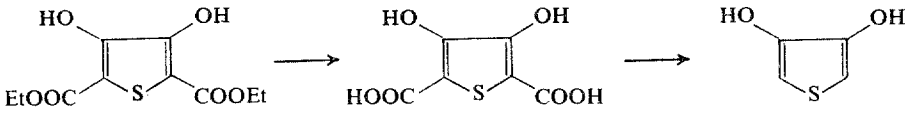
[3] C. G. Overberger and Lal Joginder, *J. Am. Chem. Soc.* **73**, 2956 (1951).



Disodio-Dicetol VII on interaction with ethyl iodide gave 2,5-dicarbethoxy-3,4-diethoxythiophene (VIII) which on hydrolysis yielded 2,5-dicarboxy-3,4-diethoxythiophene (IX). Another derivative of Dicetol prepared was 2,5-dicarboxy-3,4-ethylenedioxythiophene (X) obtained by interaction of Dicetol and ethylene dibromide in presence of potassium carbonate[4] and hydrolysis of the resulting diethyl ester X.

Hartough[5] has discussed the difficulty of saponification of Dicetol VI without concomitant decarboxylation. He has recommended the method by Turnbull[6] in which Dicetol VI was hydrolysed by fusion with sodium acetate dihydrate and sodium hydroxide at 110°. Whereas repetition of this procedure did not yield a definite product, hydrolysis of Dicetol with ethanolic sodium hydroxide gave the desired dicarboxylic acid (XII) in 80% yield. The acid darkens on exposure to light.

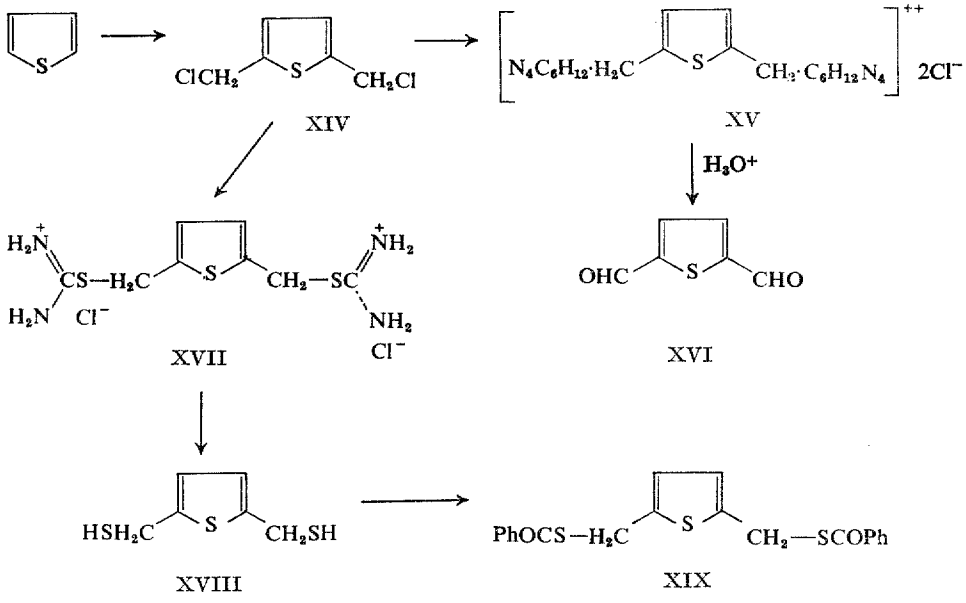
[4] B. H. Iyer and P. C. Guha, *J. Ind. Inst. Sc.*, **A21**, 115 (1938).
[5] H. D. Hartough, *Thiophene and its derivatives* p. 411. Interscience, N.Y. (1952).
[6] S. G. Turnball, (Jr) USP, 2453103; *Chem. Abstr.* **43**, 2236 (1948).



VI XII XIII

Decarboxylation of XII by heating with pyridine according to Turnbull[7] was unsuccessful. 3,4-Dihydroxythiophene (XIII) was obtained in 82% yield by heating XII at 120°/3 mm, when XIII sublimed as a yellow powder. 3,4-Dihydroxythiophene (XIII) decomposes readily on exposure to heat, light and moisture.

Among other bifunctional derivatives of thiophene, which were prepared, was 2,5-dichloromethylthiophene[8] (XIV) which served as a starting material for the synthesis of other disubstituted thiophenes. Interaction of XIV with hexamethylene tetramine (Sommelet reaction)[9] gave the hexamine salt XV which on hydrolysis gave thiophene-2,5-dialdehyde (XVI) in low yield (8·5%). Thiophene-2,5-dimethylenyl-thiouronium dichloride (XVII) was formed in quantitative yield when XIV was reacted with thiourea. Hydrolysis of XVII with aqueous sodium hydroxide gave 2,5-dimercaptomethylthiophene (XVIII) which was characterized as the dibenzoyl derivative XIX.



XIV XV XVII XVI XVIII XIX

Anticancer activity of the above compounds has been studied and a summary of the results has been published recently.[10,11] Of the various compounds described,

[7] S. G. Turnbull (Jr) USP, 2453103; *Chem. Abstr.* **43**, 2237 (1948).

[8] J. M. Griffing and L. F. Salesbury, *J. Am. Chem. Soc.*, **70**, 3316 (1948).

[9] S. J. Angyal, *Organic Reactions* Vol. VIII; p. 197. Wiley, N.Y. 197 (1954).

[10] M. B. Sahasrabudhe, M. V. Nerurkar, L. B. Kotnis, B. D. Tilak, L. G. Shah and V. N. Gogte. *Acta Union Internationale Centre le Cancer* **20**, 221 (1964).

[11] B. D. Tilak, V. N. Gogte, M. B. Sahasrabudhe and K. N. Gadekar, *Heterocyclic compounds of physiological interest* Symposium, University Grants Commission, India, (1964).

only the dithiol XVIII and the dithiouronium salt XVII proved active against Yoshida sarcoma. Treatment of rats infected with Yoshida sarcoma (ascites) cells by administration of XVII for only ten days led to a complete destruction of the malignant cells and the animals lived their normal life span. The compound XVII was however toxic when given by subcutaneous injection to Swiss mice infected with transplanted fibrosarcoma. The activity of the dithiol XVIII was much lower than XVII.

EXPERIMENTAL

Thiophene-2,5-dicarboxylic acid dihydrazide (III). A mixture of hydrazine hydrate (2 ml) and 2,5-dicarbethoxythiophene (1·0 g) was heated under reflux for 20 min. MeOH (20 ml) was added and the mixture was further refluxed for 2 hr, when a yellow ppt separated. The product was collected and crystallized from dil MeOH as pale yellow plates, (0·6 g; yield 70%) m.p. 262–263°. (Found: C, 36·4; H, 4·4. $C_6H_8N_4O_2S$ requires: C, 36·0; H, 4·0%.)

Diethyl thiodiglycollate (V) was prepared according to Becker and Stevens.[12]

2,5-Dicarbethoxy-3,4-dihydroxythiophene (VI). The compound was prepared by the Hinsberg condensation of diethyl thiodiglycollate and diethyl oxalate in presence of NaOEt.[3] By using an anchor stirrer in a round bottomed flask which ensured a thorough mixing of the reactants, 90% yield of VI was obtained (reported yield,[3] 78%). Thus interaction of diethylthiodiglycollate (20·6 g) and diethyl oxalate (14·6 g) gave VI (23·6 g; yield 90%), m.p. 135–136°, lit.,[3] m.p., 135°. (Found: C, 45·9; S, 12·3, $C_{12}H_{12}O_6S$ requires: C, 46·1; S, 12·3%.)

Disodium salt of 2,5-dicarbethoxy-3,4-dihydroxythiophene (*Disodio Dicetol*) (VII). A soln of Na (4·6 g) in abs EtOH (60 ml) was rapidly added to a well stirred soln of VI (26 g) in abs EtOH (300 ml). An exothermic reaction took place and the yellow coloured Na salt separated out immediately. The mixture was heated under reflux on a steam bath for 1 hr under stirring. After cooling to room temp, the mixture was filtered (filtration was very slow). The yellow coloured Na salt was washed with abs EtOH, till the filtrate was free of alkali and then with dry ether. The product was dried overnight in a vacuum desiccator, powdered and finally dried at 90–95°, 27·5 g, yield, 90%. (Found: C, 38·7; H, 3·1. $C_{10}H_{10}O_6SNa_2$ requires: C, 39·4; H, 3·2%.)

2,5-Dicarbethoxy-3,4-diethoxythiophene (VIII). A mixture of finely powdered VII (10 g) and Et_2SO_4 (24 ml) was heated in an oil bath at 102° for 1 hr. Excess of Et_2SO_4 was removed by distillation under vacuum and the residue was then stirred for 5 min with 5% Na_2CO_3aq (100 ml) and cooled in the refrigerator. The sticky solid obtained was filtered off, dissolved in ether and the ether soln washed with water and dried (Na_2SO_4). The product obtained on removal of ether gave on distillation, a yellow liquid b.p. 150–160°/0·6 mm. (3·0 g; yield 30%), which sets to a waxy solid on keeping. (Found: C, 53·4; H, 6·4; S, 10·3. $C_{14}H_{20}O_6S$ requires: C, 53·2; H, 6·4; S, 10·1%.)

2,5-Dicarboxy-3,4-diethoxythiophene (IX). A mixture of VIII (3·0 g) and 25% NaOHaq (25 ml) was heated under reflux for 1 hr. The mixture was cooled and acidified with 1:1 HClaq, when a white solid was obtained which crystallized from water in white needles, m.p. 245–250°, 1·6 g; yield 64%. (Found: C, 46·2; H, 4·7; S, 12·1. $C_{10}H_{12}O_6S$ requires: C, 46·2; H, 4·6; S, 12·3%.)

2,5-Dicarbethoxy-3,4-ethylenedioxythiophene (X) was prepared according to Iyer and Guha.[4]

2,5-Dicarboxy-3,4-ethylenedioxythiophene (XI). The above X (6·7 g) was refluxed with 10% NaOHaq (20 ml) for one hour. The mixture was filtered and the filtrate acidified when a white solid was obtained. The product crystallized from MeOH in colourless needles m.p. 323° (dec), 3·9 g; yield, 73%. (Found: C, 41·5; H, 2·6. $C_8H_6O_6S$ requires: C, 41·7; H, 2·6%.)

2,5-Dicarboxy-3,4-dihydroxythiophene (XIII). Dicetol VI (1·0 g) was dissolved in EtOH (30 ml) and to this soln NaOH (4·0 g) in water (20 ml) was added and the mixture was refluxed for 16 hr. EtOH was removed by distillation under vacuum and the residue was redissolved in water, the soln acidified with 15% HClaq when a white ppt was obtained. The product was collected, washed with cold water (20 ml) and then crystallized from dil MeOH when it gave XII as colourless needles (0·65 g, yield, 81%) m.p. 192° dec. (lit.,[6] m.p. 190° dec). The compound XII gave a deep blue colour with $FeCl_3$aq. (Found: C, 35·5; H, 2·0. Neutralization equiv 108. Calc for $C_6H_4O_6S$: C, 35·3, H, 2·0%. Neutralization equiv 102.) The compound darkened on exposure to air.

[12] H. J. Becker and W. Stevens, *Rec. Trav. Chim.* **59**, 435 (1940); *Beilstein* **3**, 257.

3,4-*Dihydroxythiophene* (XIII). The acid XII (1·0 g) was sublimed in a sublimation tube at 120°/3 mm. The yellowish coloured decarboxylated product was resublimed and then crystallized from benzene and ligroin when it gave pale yellow needles (0·47 g, yield 82%) m.p. 90–91° (lit.,[7] 90–91·5°). The compound darkened on standing.

2,5-*Dichloromethylthiophene* (XIV) was prepared according to Griffing and Salisbury,[8] b.p. 105–107°/2 mm.

Thiophene-2,5-*dialdehyde* (XVI). Compound XIV (27 g) was added to a boiling soln of hexamethylenetetramine (42 g) in Chf (300 ml) when a reddish brown compound separated. The reaction mixture was heated under reflux for 3 hr more, cooled and filtered. A brown coloured hexamine salt XV was obtained. The latter was added to 50% AcOHaq (500 ml) and the mixture was heated under reflux for 8 hr. Conc HCl (50 ml) was added and heating continued further for 1 hr. AcOH was removed by distillation under vacuum and the residue extracted several times with ether (7 times/50 ml). The ether extract was washed with sat $NaHCO_3$aq and then with water. On removal of ether, a greenish yellow compound was obtained which crystallized from ligroin (b.p. 60–80°) in pale yellow flakes (1·8 g; yield 8·5%) m.p. 118–119°. (Found: C, 51·4; H, 2·8; S, 22·8. $C_6H_4O_2S$ required: C, 51·4; H, 2·9; S, 23·0%.)

Thiophene-2,5-*dimethyleneyl-thiouronium dichloride* (XVII)

A hot soln of thiourea (3·08 g) in abs EtOH (100 ml) was added dropwise to a soln of XIV (3·6 g) in benzene (75 ml). The mixture was heated under reflux for 4 hr when a brown coloured crystalline product separated out. After removal of the solvents, the residue was triturated twice with benzene (25 ml). The product crystallized from water in pale yellow needles (6·0 g; yield 90%) m.p. 220° dec. (Found: N, 16·2. $C_8H_{14}Cl_2N_4S_3$ requires: N, 16·8%.)

2,5-*Dimercaptomethylthiophene* (XVIII). A soln of XVII (1·0 g) in water (10 ml), 10% NaOHaq (25 ml) and EtOH (25 ml) were heated together under reflux for 1 hr. After removal of aqueous EtOH under vacuum the residue was redissolved in water (25 ml). The soln was acidified and extracted with ether. Removal of ether gave XVIII which was purified by repeated vacuum distillation, b.p. (bath temp) 120–121°/2 mm colourless liquid, 0·43 g; yield 81%. (Found: C, 41·6; H, 4·3. $C_6H_8S_3$ requires: C, 40·9; H, 4·6%.)

2,5-*Dibenzoylmercaptothiophene* (XIX). Compound XVII (1·0 g) was dissolved in water (10 ml) and 10% NaOHaq (25 ml) was added under stirring under N atm. After 1 hr, benzoyl chloride (2 ml) was added under vigorous stirring. The reaction mixture was stirred at room temp for 2 hr more and then extracted with ether. The ether extract gave XIX which crystallized from MeOH in colourless needles (0·5 g; yield, 43%) m.p. 89–90°. (Found: C, 62·9; H, 3·9. $C_{20}H_{16}O_2S_3$ requires: C, 62·5; H, 4·2%.)

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