## 399. *Synthesis of β-2-Thienylalanine and of β-2-Thienylethylamine.*

By GEORGE BARGER and ALEXANDER P. T. EASSON.

Both substances were synthesised from thiophen-2-aldehyde, the *amino-acid* by the hippuric acid and by the hydantoin methods, the amine by the Hofmann degradation of β-2-*thienylpropionamide*. The amine has a pressor action qualitatively and quantitatively indistinguishable from that of β-phenylethylamine, which finding is attributed to the similarity in physical properties of the two bases. Thienylalanine, not being a constituent of the animal body, is not so completely oxidised as is phenylalanine.

Experience with a simple apparatus for the cheap production of thiophen from acetylene and pyrites is recorded.

WHILST comparison is often made between chemical constitution and pharmacological action, the latter in many cases depends in the first place on the physical rather than on the chemical properties of the drug. Thus the great similarity in the physical properties of corresponding benzene and thiophen derivatives leads to a close agreement in pharmacological properties, as in the case of cocaine and its thiophen analogue (Steinkopf and Ohse, *Annalen*, 1924, **437**, 14). The same was found for thiophen derivatives corresponding to eucaine, stovaine and other drugs (*idem*, *ibid.*, 1926, **448**, 205). In all these cases a 2-thienoyl group replaces benzoyl as a subsidiary substituent in a large molecule. In order to extend the comparison to substances in which the two rings constitute the principal portion of the molecule, we have prepared β-2-*thienylalanine* and its decarboxylation product, corresponding respectively to phenylalanine and β-phenylethylamine.

On account of its high price, we made the necessary thiophen from acetylene and pyrites. Our apparatus was similar to that designed by Steinkopf and Kirchoff (*Annalen*, 1914, **403**, 1) and was constructed in the laboratory, but, being simpler and smaller, gave a much poorer yield; nevertheless we could prepare large amounts of thiophen at one-tenth of the commercial price. Direct conversion into thiophenaldehyde failed entirely by Gattermann's carbon monoxide method (*Annalen*, 1906, **347**, 347). Reduction of thienoyl chloride by Rosenmund's method (*Ber.*, 1918, **51**, 585) yielded only traces of the aldehyde and the reaction between thienylmagnesium bromide and methyl *iso*formanilide (Gattermann, *Annalen*, 1912, **393**, 215) gave a yield of 20—25% of the bromothiophen employed. We ultimately found the much older and more circuitous route of Biedermann (*Ber.*, 1886, **19**, 636) the best, *via* 2-thienyl methyl ketone and 2-thienylglyoxylic acid.

From the aldehyde, β-2-thienylalanine was first prepared by Erlenmeyer's hippuric acid method, and then much more readily by the hydantoin method devised by Wheeler and Hofmann (*Amer. Chem. J.*, 1911, **45**, 368) for phenylalanine and tyrosine.

The synthesis of β-2-thienylethylamine was first attempted *via* thienylmethyl chloride; the chloromethyl group has been introduced into the benzene ring by Stephen, Short, and Gladding (J., 1920, **117**, 510) and, *via* a Grignard reagent, by Ziegler (*Ber.*, 1921, **54**, 737),

but neither of these methods yielded us thienylmethyl chloride, and the process of Biedermann (*loc. cit.*) also was unsatisfactory. The condensation of thiophenaldehyde with nitromethane was not promising. The desired amine was, however, readily obtained from the aldehyde by a method analogous to that of Haworth, Perkin, and Rankine (J., 1924, **125**, 1693) for β-3 : 4-methylenedioxyphenylethylamine.

β-2-Thienylethylamine proved to be very similar to β-phenylethylamine in physical properties and Prof. J. H. Burn found the pressor action of the two amines to be indistinguishable both qualitatively and quantitatively. Since this action can be measured with greater accuracy than most biological reactions (a discrimination of 5% is possible in the case of adrenaline), we may infer, in this case even more than in the above-mentioned analogues of cocaine, etc., that the pharmacological properties depend in the first place on the physical ones.

Whilst the physical resemblance between β-2-thienylalanine and phenylalanine is also considerable, the latter is a constituent of the animal body and is peculiar among benzene derivatives in being completely oxidised. Preliminary experiments with 0·5 g. of thienylalanine injected intravenously into a rabbit resulted within 2 hours in the appearance of a persistent ninhydrin reaction in the urine, but we were unable to obtain any thiophen derivative from it, although after oral administration of thienylcarboxylic acid we could isolate the latter partly as thienoylglycine (Jaffe and Levy, *Ber.*, 1888, **21**, 3458) and partly unchanged.

## Experimental.

*Preparation of Thiophen.*—This was carried out in the Technical Chemistry Department of Edinburgh University, and we are indebted to the late Allin Cottrell for help and advice. An iron tube, 76 × 7·6 cm., was fitted with caps through which passed a coaxial rod fitted with spirally placed vanes to act as a stirrer. The tube was surrounded successively with asbestos paper, a coil of nichrome wire, and a lagging made from magnesium carbonate and asbestos fibre. A current of 4·5 amp. from 230-volt a.c. mains kept the tube at 300°, measured by an internally placed thermometer. The outlet led to a cross piece of four pipes with clearing rods, as used by Steinkopf and Kirchoff (*loc. cit.*). The acetylene, obtained from cylinders, was passed through water to remove acetone and then dried in calcium chloride towers. The pyrites, kindly presented by Messrs. J. and J. Cunningham of Leith, contained 46·8% of sulphur and 2·8% of moisture; it was practically free from arsenic. After being ground for an hour or so in a ball mill, it almost completely passed a sieve of 90 meshes to the inch, and was mostly used unsifted. In the original form of the apparatus the charge of pyrites could be renewed only by dismantling the tube, so we soon introduced side pieces in the terminal caps which enabled a charge to be replaced by rotation of the stirrer; the charge was uniformly distributed by moving the stirrer forward and backward an equal number of turns. A slow current of carbon dioxide was passed during the preliminary heating, and when the temperature reached 260° and the pyrites had been dried, carbon dioxide was replaced by acetylene: the temperature reached 300° in about an hour. The issuing gases passed through a vertical condenser into a Woulff's bottle and out through a second condenser into a " compo " coil cooled in ice; since there was no condensate here, the coil was dispensed with. The gases then went through a 4 ft. horizontal tube containing porcelain chips soaked in paraffin oil, and were finally burnt. In the best experiment 707 l. of acetylene were passed at 300° over 2·27 kg. of pyrites (90 mesh) during 10 hours and gave 310 c.c. of distillate. At 325° the yield of distillate was slightly less than at 300°, and with short runs of 3—4 hours and a slower current of acetylene the yield was much less. Doubling the rate of the acetylene current diminished not only the relative but also the absolute yield of distillate. The amount of distillate from the paraffin scrubber was less than one-tenth of the whole. Our maximum efficiency was 43·8 c.c. of distillate per 100 l. of acetylene, as compared with 112 c.c. in Steinkopf and Kirchoff's experiments. Their apparatus could deal with 170 l. of acetylene per hour and the tube (we were informed by Prof. Steinkopf) was 2 m. long. We think that its greater efficiency may have been due to the superior stirrers employed, consisting of a continuous spiral. At the same time, we conclude that it is necessary to renew the surface of the pyrites from time to time only, by occasional stirring. As in Steinkopf and Kirchoff's experiments, our distillate gave on fractionation about 40% of thiophen. The cost of the thiophen prepared was about 55/- per kg. for acetylene and heating energy. A separate experiment showed that pyrites passing a 30-mesh sieve gave less than half the yield of that passing the 90 mesh. No improvement was effected by mixing the acetylene with half

a volume of dry hydrogen sulphide, except towards the end of a long experiment, when the charge was partly exhausted.

*Preparation of Thiophen-2-aldehyde.*—(a) *Gattermann's method.* In two of several experiments thiophen (12 g.) in benzene or light petroleum (180 c.c.), with cuprous chloride (2·5 g.), became warm on addition of aluminium chloride (22 g.); a viscous mass separated, which became more mobile on passage of a mixture of carbon monoxide and hydrogen chloride. After extraction with bisulphite, only a trace of oil was obtained, having a faint odour of thiophenaldehyde. Failure was probably due to the decomposition of thiophen by aluminium chloride. (b) *Rosenmund's method.* A mixture of thienoyl chloride (Jones and Hurd, *J. Amer. Chem. Soc.*, 1921, **43**, 2444) (9 g.) in toluene (55 c.c.), with unreduced barium sulphate–palladium catalyst (4 g.), was treated at the b. p. with a current of hydrogen. Thiophenaldehyde was obtained in a yield of 20%. In addition an ester of thiophencarboxylic acid was formed. Its b. p. and the absence of the indophenin reaction suggested an aliphatic alcohol (perhaps *n*-butyl) rather than thienylmethanol. Rosenmund's method might be turned to better account by reducing the activity of the catalyst. (c) We were thus obliged to follow the route outlined by Biedermann. 2-Thienyl methyl ketone was prepared in a yield of 60—82%; gradual addition of the solution of thiophen and acetyl chloride to the aluminium chloride under light petroleum gave rather better results than the reverse way, adopted by Biedermann. In the next step, oxidation to thienylglyoxylic acid, the concentration of his very dilute solution could safely be doubled, but a tenfold increase diminished the yield to half, favouring the formation of thienylcarboxylic acid. For the decarboxylation of thienylglyoxylic acid we found heating in glacial acetic acid at 200° for 2 hours much better than heating to the same temperature in naphthalene or diphenylmethane, or than the dry distillation employed by Biedermann. The average overall yield of aldehyde from ketone was 45%.

*Synthesis of* β-2-*Thienylalanine.*—(a) *With hippuric acid.* The first two stages followed closely the synthesis of *r*-2-methyltryptophan (Barger and Ewins, *Biochem. J.*, 1917, **11**, 60). The *azlactone* of *α-benzamido-β-2-thienylacrylic acid* formed fine yellow needles from alcohol–chloroform, m. p. 175°; yield, 70% (Found: S, 12·6. $C_{14}H_9O_2NS$ requires S, 12·6%). The *acid* formed colourless crystals from alcohol, m. p. 238—240°; yield, quantitative (Found: S, 11·7; equiv., 269, 271. $C_{14}H_{11}O_3NS$ requires S, 11·7%; equiv., 273). The simultaneous hydrolysis and reduction of the acid (as carried out by Barger and Ewins, *loc. cit.*) proved to be unsatisfactory in the present case; ammonia was evolved. Hence α-benzamido-β-2-thienylacrylic acid, dissolved in sodium hydroxide solution, was first reduced by sodium amalgam (3%), disappearance of the reddish-brown colour indicating the completion of the reaction. When the alkalinity was kept down merely by repeated addition of hydrochloric acid, no crystalline product could be obtained, but when carbon dioxide was used, and a small quantity of a neutral oil, b. p. 195°, had been removed by ether, the solution after acidification deposited overnight *α-benzamido-β-2-thienylpropionic acid*, which formed crystals from alcohol, m. p. 176—180°; yield, 24% (Found: equiv., 279. $C_{14}H_{13}O_3NS$ requires equiv., 275). This acid was hydrolysed by boiling with 10% hydrochloric acid for 6 hours. The cooled solution was washed with ether, to remove benzoic acid, and was evaporated to dryness. On sublimation of the residue in a high vacuum and crystallisation from alcohol β-2-*thienylalanine* was obtained in thin leaflets, m. p. 274—275° (bath preheated to 270°), soluble in 40 parts of water at 15°, much more soluble in dilute aqueous ammonia; the substance did not give the indophenin reaction (Found: C, 49·0; H, 5·3; N, 7·8; S, 18·3. $C_7H_9O_2NS$ requires C, 49·1; H, 5·3; N, 8·2; S, 18·7%). Since the yields in the last two reactions were low, we had recourse to another method, which obviated the removal of a benzoyl group, and proved more satisfactory.

(b) *With hydantoin.* A mixture of thiophenaldehyde (38·5 g.), hydantoin (38·8 g.) (Harries and Weiss, *Ber.*, 1900, **33**, 3418), anhydrous sodium acetate (25 g.), and acetic anhydride (145 c.c.) was heated to 110°. The solids went into solution and after ½ hour crystals began to form. After keeping overnight, the pale yellow crystals were washed with a little alcohol, ether, and water; m. p. 214—216°, yield 39·2 g. The substance is probably *acetyl-2-thienylidenehydantoin* (Found: S, 13·1, 13·2. $C_{10}H_8O_3N_2S$ requires S, 13·5%). On solution in cold aqueous sodium hydroxide and treatment with acid, *2-thienylidenehydantoin* was obtained, which formed thin leaflets from alcohol, m. p. 253—255° (Found: S, 16·0, 16·1. $C_8H_6O_2N_2S$ requires S, 16·5%). This hydantoin (13·8 g.) was suspended in 2 l. of spirit and shaken with 400 g. of 3% sodium amalgam; the alkali was from time to time partly neutralised with sulphuric acid. The sodium sulphate was filtered off, and the alcohol distilled under reduced pressure. On addition of water, *2-thienylmethylhydantoin* was obtained, m. p. 188—190° after crystallisation from alcohol–chloroform; yield, 75% (Found: S, 16·2, 15·9. $C_8H_8O_2N_2S$ requires S, 16·3%). The course

of the final hydrolysis was followed by titrating from time to time the ammonia given off (an advantage over the hippuric acid method). With 8 g. of crystalline barium hydroxide per 100 c.c., the reaction was very slow; with 50 g. per 100 c.c., the reaction was stopped when 97% of the ammonia had been given off, but the glass flask was attacked and the amino-acid was freed from a sodium salt only by conversion into the ester hydrochloride, from which it was ultimately recovered with the same m. p. as the sample obtained by method (a). The best results were obtained in a copper flask, in which 2-thienylmethylhydantoin (4·85 g.) was boiled with water (85 c.c.) and recrystallised barium hydroxide (42 g.). The barium was removed with a slight excess of sulphuric acid, and a little copper by hydrogen sulphide. The filtrate was concentrated and mixed with alcohol and ether; 2 g. (45%) of the pure amino-acid then crystallised.

*Synthesis of* β-2-*Thienylethylamine.*—A preliminary experiment on the conversion of thienylmethanol by hydrogen chloride according to Biedermann gave little promise of success. A small quantity of an oil with the odour of benzyl chloride was obtained, but the main product was a rubber-like mass, insoluble in alcohol or acetone, and only partly in chloroform. Hence we did not attempt to prepare thienylacetonitrile. The condensation of thiophenaldehyde with nitromethane yielded a bright yellow, volatile, crystalline substance, m. p. 79—83°, presumably the analogue of ω-nitrostyrene, but on account of the small yield (20%) we did not attempt to reduce it. The degradation of an amide proved more satisfactory.

Thiophenaldehyde (21 g.), malonic acid (47 g.), pyridine (90 c.c.), and piperidine (1·5 c.c.) were heated in the steam-bath for 2 hours. The solution was then boiled for 5 minutes, cooled, poured into water, and acidified, with cooling. No odour of the aldehyde was observed. The precipitated thienylacrylic acid, recrystallised from alcohol (charcoal), had m. p. 143—145°; yield, 85% (Biedermann, *Ber.*, 1886, **19**, 1855, obtained a 30% yield of a product, m. p. 138°, by means of sodium acetate and acetic anhydride). On reduction with sodium amalgam in neutral solution below 15°, thienylpropionic acid was obtained in a yield of 98%; m. p. 43—45°.

β-2-*Thienylpropionamide.*—The acid (22 g.), dissolved in chloroform, was mixed with thionyl chloride (25 c.c.) and kept for 12 hours. On pouring into a solution containing 17 g. of sodium hydroxide and 385 c.c. of concentrated aqueous ammonia the *amide* separated, and more was extracted by chloroform from the mother-liquor; yield 90%, m. p. 99—100° (Found: S, 20·3. $C_7H_9ONS$ requires S, 20·7%).

β-2-*Thienylethylamine.*—The above amide (19·5 g.) was dissolved by gentle warming in the solution obtained by passing the chlorine from potassium permanganate (7·7 g.) and hydrochloric acid into 300 c.c. of 10% aqueous sodium hydroxide. The solution was then heated at 70—75° for 1 hour. After addition of solid potassium hydroxide (77 g.) the mixture was heated at 80° for a few minutes. The resulting oil was separated, mixed with the benzene extract of the aqueous layer, and dried by refluxing with solid caustic potash. On fractional distillation 5·5 g. of the amine distilled at 200—201°, and from the fractions distilling up to 200° and at 201—215° a further quantity was isolated as hydrochloride, by addition of alcoholic hydrogen chloride to a solution in ether. After recrystallisation, 5·05 g. of amine hydrochloride were obtained, which with the free base amounts to a yield of 63% of the theoretical. β-2-Thienylethylamine had b. p. 200—201°/750 mm. and $d^{15°}$ 1·087 (β-phenylethylamine, b. p. 198°; $d^{24°}$ 0·958). When exposed to air, it rapidly formed a solid carbonate. The *hydrochloride* formed prisms from dilute alcohol, m. p. 200—202° (Found: S, 19·7; Cl, 21·7. $C_6H_9NS,HCl$ requires S, 19·5; Cl, 21·7%).

The cyclisation of the amine, by the methods used by Becker and Decker (*Annalen*, 1913, **395**, 342) to obtain tetrahydro*iso*quinoline derivatives from β-phenylethylamine, was unsuccessfully tried on a small scale. Treatment of thienylethylamine with formaldehyde and hydrochloric acid gave a red semi-solid, and heating the amine hydrochloride with formaldehyde alone at 130° gave a base which formed an amorphous solid picrate (the picrate of thienylethylamine is liquid). Formylthienylethylamine, b. p. 187—189°/20 mm., heated with phosphorus pentachloride or pentoxide in boiling toluene or on the water-bath, gave a minute quantity of a colourless solid base. β-2-Thienylethyltrimethylammonium iodide had m. p. 236—238°. 3 : 4-Methylenedioxybenzylidenethienylethylamine formed prisms from alcohol, m. p. 57—58°.

iso*Nitrosoacetothienone.*—This was prepared from acetothienone (12·6 g.) by the method used by Claisen and Manasse (*Ber.*, 1887, **20**, 2194) for the acetophenone derivative. Yield, 40%. It formed crystals from chloroform, m. p. 110—111°. On reduction with stannous chloride, as in the case of the corresponding benzene derivatives (Rupe, *Ber.*, 1895, **28**, 254), there was obtained *2-thienyl aminomethyl ketone hydrochloride*, m. p. 215—218°; yield, 48%

(Found : Cl, 20·1. $C_6H_7ONS,HCl$ requires Cl, 20·0%). The m. p. is more than 30° higher than that of the aminoacetophenone hydrochloride, an unusually large difference; the substance moreover melted to a blood-red liquid, which almost at once resolidified and decomposed at 235° without melting again. This behaviour may indicate the formation of a new substance, and the instability of the free amino-ketone made us abandon attempts to reduce it to an alkamine of the adrenaline type.

For further experimental details, particularly on the preparation of thiophen, see the Ph.D. dissertation by A. P. T. Easson, Edinburgh, 1930.

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Department of Medical Chemistry, University of Edinburgh.

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