## 742. *The Sex-attractant of the Silkworm Moth* (Bombyx mori).

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The sex-attractant of the female silkworm moth has been identified as dimethylamine. It has been isolated from the sacculi lateralis as *p*-*p*′-nitrophenylazobenzoyl derivative which on hydrolysis gives an attractant solution. A chromatographic procedure is described for separation of mixtures of simple primary and secondary amines as *p*-*p*′-nitrophenylazobenzoyl derivatives.

Female silkworm moths (*Bombyx mori*), soon after hatching, develop two sacks (sacculi lateralis) at the tip of the abdomen.[1] These sacks contain a material which acts as a powerful attractant for male moths.[2] After early work on different moths had been

[1] Freiling, *Wissensch. Zool.*, 1909, 92.
[2] Fabre, "Social life in the insect world," translated by B. Miall, Pelican, London, 1943; Flaschenträger and Amin, *Nature*, 1950, **165**, 394; *Angew. Chem.*, 1949, **61**, 252; Götz, *Experientia*, 1951, **7**, 406.

ineffective,[3] Butenandt [4] hydrolysed the material in the sacks from 313,000 silkworm moths, converted the non-acidic portion into *p*-*p*′-nitrophenylazobenzoyl derivatives and by counter-current fractionation and chromatography obtained material, m. p. 58—68°, giving on hydrolysis a product which was active as a sex-attractant in amounts as low as $10^{-11}$ g. But he did not identify this active product.

The present author extracted the sacculi lateralis of 3428 female moths with light petroleum, without prior hydrolysis, warmed the resultant solution with *p*-*p*′-nitrophenylazobenzoyl chloride, pyridine, and benzene, concentrated the solution, removed the pyridine and free acid, and then by chromatography, partition chromatography, and crystallisation, obtained 13·5 mg. of a pure derivative, m. p. 178°, which on hydrolysis gave a solution which was highly attractant to male moths.

The simple alkyl *p*-*p*′-nitrophenylazobenzoates had been prepared by Amin and Hecker, who also devised a chromatographic technique for their separation.[5,6] However, none of these esters resembled the materials isolated from silkworm moths. This work has now been extended to *p*-*p*′-nitrophenylazobenzoyl derivatives of ten simple primary and secondary amines. The derivative of dimethylamine is identical with the compound, m. p. 178°.

Dimethylamine is therefore the sex-attractant of the female silkworm.

In the Experimental section is described also a partition-chromatographic method of separating the amines by means of their *p*-*p*′-nitrophenylazobenzoyl derivatives on kieselguhr impregnated with dimethyloxosilane.

## Experimental

Evaporations were under reduced pressure at 50°. M. p.s were determined on the Kofler microscope stage.

*Substituted* p-p′-*Nitrophenylazobenzamides.*—(a) *Preparation.* The amine (0·5 millimole) and *p*-*p*′-nitrophenylazobenzoyl chloride [6] (0·725 millimole) were heated in 1 : 1 pyridine–benzene (14 ml.) at 50° for 6 hr. The mixture was then treated with water and extracted with benzene (50 ml.). The extract was washed with 20% sulphuric acid, filtered, washed successively with water, sodium carbonate solution, and water, concentrated to *ca.* 10 ml., and filtered through a column of alumina. The main, lower, red band was eluted with benzene, and the product recrystallised (forming needles) from ethanol, acetone, nitromethane, nonane, decane, or dimethylformamide. The p-p′-*nitrophenylazobenzoyl derivatives* recorded in the Table were thus obtained in 94—98% yield.

(b) *Hydrolysis.* The amine in benzene or *cyclo*hexane was warmed with 1 equiv. of potassium hydroxide in 2-methoxyethanol for 2 hr. at 50°. Potassium *p*-*p*′-nitrophenylazobenzoate was then filtered off, leaving the amine in solution.

(c) *Chromatography.* Kieselguhr (0·5 kg.) was dried at 110° and placed in a desiccator over dichlorodimethylsilane for 3 days, being occasionally shaken. The kieselguhr was then treated with water (1·5 l.), and the floating material was collected, washed with methanol to neutrality, and dried at 110°.

A 20 : 1 (v/v) mixture of 50% (v/v) aqueous acetone and chloroform was shaken and the two layers were separated. A Pyrex chromatography tube (*ca.* 40 cm. long and 2·0 cm. in diameter) was half-filled with the acetone layer, then a 1 : 1 (w/w) mixture of the chloroform layer and the treated kieselguhr was added, 15 g. at a time, with slight suction, air being removed by manipulation of a plunger. When the column had drained, a solution of the acylamine (≯15 mg.) in the acetone layer was added, and the chromatogram was developed with further quantities of the acetone layer. The colour in successive fractions was determined electrophotometrically.

Alternatively, 45% of 65% (v/v) aqueous dimethylformamide was shaken with carbon

[3] Amin, Thesis, Alexandria, 1949, 1952; Haller, Acree, and Potts, *J. Amer. Chem. Soc.*, 1944, **66**, 1659; Acree, *J. Econ. Entomol.*, 1953, **46**, 313.
[4] Butenandt, *Naturwiss. Rundschau*, 1955, **8**, 457.
[5] Amin and Hecker, *Chem. Ber.*, 1956, **89**, 695.
[6] Hecker, *Chem. Ber.*, 1955, **88**, 1666.

tetrachloride, the carbon tetrachloride layer being mixed with the kieselguhr, and the aqueous dimethylformamide layer used as solvent and eluant.

| Amine | Colour | Solvent | M. p. | Found (%) C | H | N | Formula | Required (%) C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|
| $NH_2Me$ | Red | $MeNO_2$ | 233° | — | — | — | — | — | — | — |
| $NH_2Et$ | Red | EtOH | 220 | 60·2 | 4·8 | 19·1 | $C_{15}H_{14}O_3N_4$ | 60·4 | 4·7 | 18·8 |
| $NH_2Pr^n$ | Orange | $MeNO_2$ | 187 | 61·2 | 5·4 | 17·7 | $C_{16}H_{16}O_3N_4$ | 61·5 | 5·2 | 17·9 |
| $NH_2Bu^n$ | ,, | $COMe_2$ | 197 | 62·6 | 5·55 | — | $C_{17}H_{18}O_3N_4$ | 62·6 | 5·6 | — |
| *cyclo*Hexylamine | ,, | ,, | 248 | 64·3 | 5·9 | 15·4 | $C_{19}H_{20}O_3N_4$ | 64·75 | 5·7 | 15·9 |
| *iso*Pentylamine | ,, | ,, | 168 | — | — | — | — | — | — | — |
| $CH_2Ph{\cdot}NH_2$ | Red | EtOH | 236 | 66·8 | 4·5 | — | $C_{20}H_{16}O_3N_4$ | 66·7 | 4·5 | — |
| $NHMe_2$ | Orange-red | $C_9H_{20}$ | 178 | 60·3 | 4·6 | 19·1 | $C_{15}H_{14}O_3N_4$ | 60·4 | 4·7 | 18·8 |
| $NHEt_2$ | Orange | $C_{10}H_{22}$ | 162 | 62·1 | 5·3 | 17·0 | $C_{17}H_{18}O_3N_4$ | 62·6 | 5·5 | 17·2 |
| $NHBu^n_2$ | ,, | EtOH | 130 | — | — | — | — | — | — | — |

Suitable eluant fractions were combined and evaporated, the residue was extracted with benzene, the benzene extract in turn evaporated, and this residue crystallised from ethanol.

The following results are typical.

(i) A mixture of *p*-*p*′-nitrophenylazobenzoyl derivatives (1 mg. each) of ethylamine, propylamine, and butylamine was chromatographed as above, but on a 10 × 2 cm. column, with the aqueous-acetone–chloroform system. Eluant fractions taken were: nos. 1—21, 2 ml.; nos. 22—31, 4 ml.; nos. 32—42, 6 ml. each. Fractions 1—15 yielded 0·8 mg. of ethylamide; fractions 16—31 yielded 0·6 mg. of propylamide; fractions 32—42 yielded 0·6 mg. of butylamide. These amides were identified by m. p.s and mixed m. p.s.

(ii) When the above mixture was chromatographed with the 45% aqueous-dimethylformamide–carbon tetrachloride system, the photometric readings showed a trace of overlap between the ethylamide and propylamide but a gap of 10 fractions between the propylamide and butylamide.

(iii) Use of the 65% aqueous-dimethylformamide–carbon tetrachloride system separated the dimethylamide from the diethylamide with a gap of two fractions.

*Isolation of the Attractant.*—Pupæ of 5000 silkworms were removed from the cocoons and the females separated. After hatching, the tips of the abdomen of 3428 female moths were cut off and placed at once under light petroleum (b. p. 50—60°) (500 ml.). The collected tips were crushed with sand and re-extracted with light petroleum (2 l.). The combined petroleum extracts were dried ($Na_2SO_4$), mixed with dry benzene (1 l.), *p*-*p*′-nitrophenylazobenzoyl chloride (0·5 g.), and pyridine (10 ml.), and heated under reflux at 50° until the solution was no longer attractant to male moths (6 hr.). The solution was concentrated to 250 ml., washed with 20% sulphuric acid, filtered, washed in turn with water, sodium carbonate solution, and water, and placed on an alumina column. Of the two coloured bands produced, the lower, red band was eluted with benzene, giving 18·54 mg. of material which on hydrolysis gave a solution attractant to male moths. The upper band, on elution with benzene, gave a non-attractant orange oil (76·9 mg.).

The attractant material (17·5 mg.) was subjected to partition chromatography on treated kieselguhr, as described above, but the decane–nitromethane system was used, giving two bands. Elution of the main, lower band with the nitromethane layer and recrystallisation of recovered material from decane gave orange-red needles (13·5 mg.), m. p. 178° alone or mixed with *NN*-dimethyl-*p*-*p*′-nitrophenylbenzamide (Found: C, 60·7; H, 4·6; N, 18·9. $C_{15}H_{14}O_3N_4$ requires C, 60·4; H, 4·7; N, 18·8%). Partition chromatography of this product with the 65% aqueous-dimethylformamide–carbon tetrachloride system gave no indication of heterogeneity.

The upper, pale orange band afforded cholesteryl *p*-*p*′-nitrophenylazobenzoate (1 mg.), m. p. 180°, which was not attractant after hydroysis.

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[*Received, April 8th*, 1957.]