amide, m. p. 99–102°. The ether-insoluble residue which composed the bulk of the original solid was crystallized twice from dilute ethanol to give the pure diamide, m. p. 156–157°.

Anal. Calcd. for $C_{12}H_{16}O_5N_2$: N, 10.44. Found: N, 10.46.

Acknowledgment.—We wish to thank Dr. Klaus Hofmann for his kindness in furnishing a sample of the dicarboxyfuranvaleric acid. Our thanks are also due to Miss Lillian Morrell for technical assistance during the course of the work.

Summary

1. A satisfactory procedure for preparing ethyl β-ketosuberate in 44% yield based on ethyl hydrogen adipate is presented.

2. A new method for preparing δ-(3,4-dicarboxy)-furanvaleric acid and some of its derivatives is described.

3. The preparation of 3,4-dicarboxyfuranacetic acid and some of its derivatives is reported.

WINTHROP CHEMICAL COMPANY, INC.
RENSSELAER, NEW YORK RECEIVED JULY 1, 1944

[CONTRIBUTION FROM UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH ADMINISTRATION, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE]

The Nature of the Sex Attractant of the Female Gypsy Moth*

BY H. L. HALLER, FRED ACREE, JR., AND S. F. POTTS

The gypsy moth (*Porthetria dispar* (L.)), one of the most serious insect pests of fruit, shade and woodland trees in New England, was accidentally introduced into Massachusetts from France in 1869 (Burgess and Baker[1]). In the last thirty to forty years approximately $1,700,000 have been spent annually for the control of this pest (Burgess[2]).

The female gypsy moth does not fly, and the male is attracted to the female by scent. As early as 1893 experiments were undertaken in which traps containing unfertilized living females were used to attract the male (Forbush and Fernald).[3] As a control measure these traps were of no value.

The area infested by the moth continued to grow, and in 1913 intensive experiments (Collins and Potts[4]) were initiated to facilitate the finding of new infestations. It was soon found that males were attracted to an extract prepared from the abdominal tips of unfertilized females, and at the present time benzene extracts of the tips are used in scouting traps. Briefly, the bait for each trap is prepared by clipping the tips from 30 unfertilized live females twenty-four to forty-eight hours after emergence, placing them in a cork-sealed bottle with 1 ounce of benzene, and storing at 35°F. until needed for use.

According to Collins and Potts,[4] chemical studies on the extractives were initiated at the Harvard University Medical School in 1925 by W. R. Bloor.[5] From experiments carried out that year, he[5] concluded that the males are attracted by the odor of a substance produced in the female abdominal tip, and that this relatively stable substance is soluble in fat solvents and slightly soluble in water, and is non-saponifiable. In 1926 the studies were continued by C. H. Fiske.[5] He suspected that the substance was an aldehyde, but he was unable to isolate it. The next year, B. L. Souther[5] working at the same school concluded that the substance was probably an indifferent saturated material—fat, protein, or ester; that it was destroyed by cold concentrated hydrochloric acid and by boiling alcoholic potash; and that it was readily volatile with solvents and continuously generated by hydrolysis of a more complex compound.

In the fall of 1941 the chemical study of the gypsy moth attractant was renewed by the Bureau of Entomology and Plant Quarantine and prior to the flight season in 1942 and 1943 experiments were performed with two lots of a benzene extract of the material. The first lot was prepared as described above from female moths collected in 1941, and the second from female moths collected in 1942. In each case the tips were separated from the benzene solution and washed with benzene. The benzene was then removed from the combined solutions and the residues were treated as described below. Each treatment resulted in one or more fractions, samples of which were tested in the field for attractiveness to male moths in comparison with an aliquot of the starting material. Each fraction was tested in five replicates at the same equivalent concentration of 30 moth tips per 30 ml. of benzene solution per trap.

Included in the tests of 1942 were several chemicals that had been shown to attract males of other species (Götz,[6] Lehman,[7] and Travis[8]) one substance known to occur in insect urine

* Not copyrighted.

(1) A. F. Burgess and W. L. Baker, U. S. Dept. Agr. Cir. 464, 28 pp. (1938).

(2) A. F. Burgess, *J. Econ. Entomol.*, **33**, 558 (1940).

(3) E. H. Forbush and C. H. Fernald, "The gypsy moth, *Porthetria dispar* (Linn.)," Mass. State Board of Agriculture, Boston, 1896, p. 345.

(4) C. W. Collins and S. F. Potts, U. S. Dept. Agr. Tech. Bull. 336 pp., 1932.

(5) Unpublished report quoted in (4) p. 14.

(6) B. Götz, *Umschau*, **44**, 794 (1940).

(7) R. S. Lehman, *J. Econ. Entomol.*, **25**, 949 (1932).

(8) B. V. Travis, *ibid.*, **32**, 690 (1939).

(Wigglesworth[9]), and some related substances. There is no indication of attractiveness to the male gypsy moth with any of these chemicals, which were as follows: cantharidin, *n*-caproic acid, isocaproic acid, salicylic acid, salicylic aldehyde, butyric acid, isoamylamine, and extracts of both Chinese and Russian cantharides. Exaltone and civetone,[10] two animal attractants, were included in the 1943 tests, but they showed no attractiveness.

The information gained from the exploratory experiments in 1942 led to a schematic chemical fractionation of the benzene extractive. The comparative attractiveness of the fractions was obtained from field tests conducted in 1943, so far as possible under the same conditions that prevailed in 1942. The results reported by Collins and Potts[4] have been confirmed with but few exceptions. The attractant is specific for the male gypsy moth. It is relatively stable and soluble in the usual fat solvents. It is not appreciably volatile at 50–60°, p = 20 mm., although it is somewhat volatile with steam. The substance contains no free acidic or basic groups, and it is found in the unsaponifiable fraction after the benzene extractive has been refluxed with dilute ethanolic potash. Under proper conditions the attractant can be partly separated by chromatographic analysis, and it can be concentrated in the petroleum ether fraction by the procedure for the separation of the pyrethrins described by LaForge and Haller.[11] When the benzene extractive is hydrolyzed with boiling dilute ethanolic hydrochloric acid, the attractant is destroyed, but the attractiveness of the extracted tips is partly regenerated by this treatment. Souther concluded that the attractant is saturated, but in this Laboratory the attractiveness of the unsaponifiable fraction has been found to be markedly increased by treatment with catalytic hydrogen. When the unsaponifiable fraction, either before or after hydrogenation, is allowed to react with phthalic anhydride in pyridine solution and the separated carbonate-soluble reaction product is hydrolyzed, a viscous residue is obtained which still retains the attractiveness of the original material. Cholesterol was isolated from the non-hydrogenated residue, but it was unattractive.

Experimental

The benzene extractive was obtained when required for the chemical treatments described below by removing the solvent (as in all other distillations) at 50–60°, p = 20 mm., in nitrogen. In the case of the bait prepared in 1941, 300-ml. aliquots (300 tips) of a stock solution were used for each treatment. Each of the various fractions recovered by the usual procedures from the chemical treatments was diluted with benzene to 300 ml. producing a test solution equivalent to 1 tip per milliliter. In the case of the bait prepared in 1942, the benzene extractive was isolated and aliquots were weighed for the treatments described below. Test samples were prepared at the equivalent of 1 tip per milliliter.

Stock Solution of Benzene Extractive from Tips Collected in 1941.—The abdominal tips from 12,000 female moths were separated from the benzene trap solutions by filtration, washed twice with benzene, and extracted with acetone. (Traps baited with aliquots of the acetone solution were unattractive to male moths.) The combined benzene solutions of tip extractive were diluted to 12 liters with benzene, providing a stock solution equivalent to 1 tip per milliliter, which was stored in an atmosphere of nitrogen at 5°. Check traps baited with aliquots of this stock solution without any chemical treatment caught 81 male moths.

Volatility of Attractant.—The benzene distillate and the tip extractive were recovered and tested. This experiment was repeated. The average catch was 5 moths with the distillate and 108 moths with the extractive.

Acidity or Basicity of Attractant.—The ether solution of the extractive was carefully separated by extraction with dilute potassium hydroxide and dilute hydrochloric acid. The neutral fraction caught 109 moths, the acidic fraction 4, and the basic fraction 3.

Steam Volatility of Attractant.—The extractive was steam-distilled until the distillation of the small quantity of oil appeared to be complete. After separation by ether extraction, the volatile fraction caught 109 moths and the non-volatile fraction 125.

Alkaline Hydrolysis of Attractant.—The extractive was refluxed for one hour with an excess of dilute ethanolic potassium hydroxide. The catch with the ethanol distilled from the reaction mixture was 5 moths. The saponification residue was separated into the acidic fraction, which caught 10 moths and the neutral fraction, which caught 117.

Acid Hydrolysis of Attractant.—The extractive was refluxed for one hour with an excess of dilute ethanolic hydrochloric acid. The ethanol recovered from the reaction mixture caught 3 moths, and the acid-insoluble residue none.

Solvent Partition of Attractant.—The extractive was dissolved in petroleum ether and partitioned with 90% acetic acid according to the procedure described by LaForge and Haller.[11] The separated petroleum ether-soluble fraction caught 119 moths and the acetic acid-soluble fraction 21.

Separation of Attractant from Fats.—The extractive was dissolved in hot 80% ethanol and allowed to stand for twenty four hours at 5°. The solid fatty fraction was then filtered and tested in comparison with the soluble fatty fraction, which was recovered by removing the solvent from the filtrate. The respective catches were 95 and 148 moths.

Hydrogenation of Attractant.—The benzene removed from an aliquot absorbed 400 ml. of hydrogen when shaken in an atmosphere of hydrogen with reduced platinum oxide catalyst. The catalyst was filtered and the benzene filtrate caught 3 moths.

The extractive obtained from this aliquot was dissolved in ethanol and absorbed 250 ml. of hydrogen when treated as just described. After the catalyst was separated, the ethanol removed from the solution caught 2 moths as compared with 178 with the hydrogenated extractive that remained.

Selective Absorption of Attractant.—The extractive dissolved in 75 ml. of a 2:1 mixture of petroleum ether and benzene was passed into a 12 × 200 mm. column of Brockmann alumina. The column was eluted with benzene, and the first and second 75-ml. portions of percolate caught 114 and 26 moths, respectively. The column was then eluted with ether; no moths were caught with the third 75-ml. portion of percolate.

Isolation of Benzene Extractive from Tips Collected in 1942.—The abdominal tips from 34,800 moths were removed from the benzene mixture in a fruit press and washed twice with benzene. The washed and pressed tips (2170 g. air-dried) caught 8 moths. The tips were hydrolyzed as described below.

The benzene solutions were separated from the water

(9) V. B. Wigglesworth, "The principles of insect physiology," E. P. Dutton Co., New York, 1939, p. 304.

(10) Furnished by L. W. Butz, Bureau of Animal Industry.

(11) F. B. LaForge and H. L. Haller, THIS JOURNAL, **57**, 1893 (1935).

solution that settled out. The water solution, after being washed twice with benzene, caught 1 moth. All the benzene solutions were combined, and the solvent was removed. The residual benzene extractive (31.5 g.) caught 71 moths.

Acid Hydrolysis of Extracted Moth Tips.—A 300-tip aliquot of the air-dried tips was refluxed for one and one-half hours with 200 ml. of ethanol containing 5% of hydrochloric acid. The tips were filtered from the cold reaction mixture and washed with ethanol. The combined ethanol solutions were diluted with water and extracted with ether. After being washed with a dilute solution of sodium bicarbonate and then with water, the ether solution was dried and the solvent was removed by evaporation. The residue attracted 59 moths.

Alkaline Hydrolysis of Extracted Moth Tips.—A 300-tip aliquot of the extracted tips was refluxed for one and one-half hours with 200 ml. of ethanol containing 5% of potassium hydroxide. The ethanol solution was cooled and filtered from the tips, which were washed with ethanol. The combined ethanol solutions were diluted with water and extracted with ether. The ether solution was washed with water and dried, and the solvent was removed. The residue caught 53 moths.

Hydrogenation and Saponification of Benzene Extractive.—A 600-tip aliquot (540 mg.) of the benzene extractive was dissolved in 25 ml. of warm ethanol and shaken in an atmosphere of hydrogen with 50 mg. of reduced platinum oxide catalyst. A small amount of colorless crystalline material separated as the hydrogenation proceeded. The reaction stopped after the absorption of 25 ml. of hydrogen. The crystalline material was dissolved by warming, and the catalyst was filtered. When the filtrate was shaken with a fresh lot of reduced catalyst, an additional 25 ml. of hydrogen was absorbed. The catalyst was filtered from the warmed reaction mixture, which was then diluted with ethanol to 50 ml. and divided into two 25-ml. portions. One portion attracted 127 moths.

The other 25-ml. portion of the ethanol solution was refluxed in nitrogen for one and one-half hours with 1 ml. of a 5% aqueous solution of potassium hydroxide. The reaction mixture was cooled, diluted with water, and extracted with ether. The ether solution was washed with water and dried, and the solvent was removed. The catch with this residue was 262 moths.

In another experiment a 1200-tip aliquot (1.08 g.) of the benzene extractive was hydrogenated as described above and absorbed 160 ml. of hydrogen after shaking with one 100-mg. lot of reduced platinum oxide. The warmed reaction mixture was separated from the catalyst and cooled. The material (222 mg.) that separated from the solution was filtered and dried. It melted at 57–59°, and after being twice recrystallized from acetone it melted at 60–60.5°. This material gave a positive test for glycerol and caught 1 moth.

Saponification of Benzene Extractive.—A 15,900-tip aliquot (14.4 g.) of the benzene extractive was refluxed in nitrogen for one hour with 100 ml. of ethanol containing 2% of potassium hydroxide. The reaction mixture was cooled, diluted with water, and extracted with ether. The ether solution was washed with water, then with 5% aqueous hydrochloric acid, and finally with water. The solvent was removed by evaporation from the ether solution after it was dried, and the waxy neutral residue (6.39 g.) attracted 121 moths. The material gave a positive Liebermann–Burchard sterol test and negative tests for nitrogen and sulfur.

The hydrochloric acid wash was made alkaline and extracted with ether. The ether solution was washed with water and dried, and the solvent was removed to yield the acid-soluble fraction. Only 1 moth was caught.

Selective Absorption of Neutral Fraction.—A 300-tip aliquot (120 mg.) of the neutral fraction dissolved in 15 ml. of benzene was added to a 10 × 200 mm. column of Alorco alumina. This solution was washed in with 25 ml. of petroleum ether and eluted with sufficient of a 2:1 mixture of petroleum ether–benzene to give 200 ml. of percolate. The percolate caught 10 moths.

Solvent Partition of Neutral Fraction.—A 300-tip aliquot (120 mg.) of the neutral fraction was dissolved in petroleum ether and separated according to the procedure of LaForge and Haller[11] into a fraction soluble in petroleum ether, which caught 132 moths, and another fraction soluble in 90% acetic acid, which attracted only 1 moth.

Hydrogenation of Neutral Fraction.—A 7500-tip aliquot (3 g.) of the neutral fraction was taken up in warm ethyl acetate and filtered from a small quantity of amorphous solid. After being shaken in an atmosphere of hydrogen with 100 mg. of reduced platinum oxide for one hour, the solution absorbed only 25 ml. of hydrogen. The reaction mixture was filtered and shaken as before with a fresh lot of reduced catalyst. After two hours, 160 ml. of hydrogen was absorbed and the catalyst was filtered from the reaction mixture. The catch with a 300-tip aliquot of the filtrate was 233 moths. The solvent was removed from the remainder of the solution and yielded 2.88 g. of residue, equivalent to 7200 tips.

Reaction of Phthalic Anhydride with the Hydrogenated Neutral Fraction.—The hydrogenated neutral fraction (2.88 g.) was mixed with 3 g. of phthalic anhydride and 10 ml. of freshly distilled pyridine in a sealed flask. After being heated on the steam-bath for twenty hours, the contents of the flask was poured into 200 ml. of 3% hydrochloric acid containing ice. The mixture was extracted with ether and washed free of mineral acid with water. The ether solution was then separated into the carbonate-soluble fraction and the carbonate-insoluble fraction. The latter (1.9 g.) attracted no moths. The carbonate-soluble fraction was refluxed in nitrogen with an excess of sodium ethylate. The alcohol fraction (0.33 g.), which was separated by solvent extraction, caught 85 moths.

Reaction of Phthalic Anhydride with Neutral Fraction.—A 7500-tip aliquot (3 g.) of the neutral fraction was treated with phthalic anhydride–pyridine mixture and worked up as described above. With the carbonate-insoluble fraction (2.06 g.) recovered by removal of the ether, 17 moths were caught. The carbonate-soluble fraction, after saponification, yielded the alcohol fraction (0.88 g.), which caught 113 moths and gave a strong Liebermann–Burchard test for sterols.

Separation of Cholesterol and Alcohols.—The 0.85 g. remaining of the foregoing alcohol fraction was digested with several portions of boiling ethanol. One moth was caught with the small quantity of tarry residue. The ethanol solution (50 ml.) was mixed with 50 ml. of hot 1% ethanol solution of digitonin. After the reaction mixture had stood overnight at room temperature, the digitonide (500 mg.) which soon began to crystallize was separated and decomposed with hot, dry pyridine according to the method of Bergmann.[12] Removal of the solvent from the ether extract of the decomposed digitonide yielded 130 mg. of residue, which when crystallized attracted 1 moth. This material was recrystallized from methanol to a constant melting point of 143–144°, which is very close to the melting point recorded for cholesterol, and it weighed 56 mg.; $[\alpha]^{29}D$ −39.6° (in chloroform).

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.89, 83.74; H, 11.96, 12.05.

When 25 mg. of this compound was boiled with acetic anhydride and then cooled, a compound crystallized, which was filtered and recrystallized from methanol. The substance weighed 23 mg. and melted at 116–117°, a temperature that has been recorded for cholesteryl acetate.

The solvent was removed from the ethanolic filtrate obtained above. The partly crystalline residue, consisting of alcohols and digitonin, was digested with several portions of dry ether and filtered. Upon removal of the ether there remained a viscous oil (240 mg.), which attracted 73 moths. This material did not respond to the Liebermann–Burchard sterol test.

Summary

Benzene extractives prepared from the abdom-

(12) W. J. Bergmann, *J. Biol. Chem.*, **132**, 471 (1940).

inal tips of virgin female gypsy moths are attractive to males. The attractiveness was markedly increased by hydrogenation. The attractant remained in the neutral fraction after saponification and it reacted with phthalic anhydride. It was recovered from the phthalic acid ester by saponification. The attractant is specific for the male gypsy moth. None of several synthetic materials tested showed any attractiveness..

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[CONTRIBUTION FROM THE AMMONIA DEPARTMENT, E. I. DU PONT DE NEMOURS & CO., INC.]

Analytical Procedures Employing Karl Fischer Reagent. XII. The Determination of Primary Amines[1]

BY WALTER HAWKINS, DONALD MILTON SMITH AND J. MITCHELL, JR.

The determination of primary amines with few exceptions has long been limited to reaction with nitrous acid, which often is not satisfactory. Occasionally the reaction between primary amine and aldehyde has been used for specific purposes, including reactions with formaldehyde,[2] for the identification of aromatic amines[3] and for the separation of primary aryl amines and secondary alkylaryl amines with benzaldehyde–sodium bisulfite.[4] Colorimetric methods for the identification of primary amines based on reaction with anhydro-bis-indanediones[5] and for monoethanolamine[6] have been reported. None of these last techniques are generally applicable to the determination of the primary amino group.

In the present investigation a technique has been developed based on the Schiff type reaction

$$RNH_2 + C_6H_5CHO \longrightarrow RN{=}CHC_6H_5 + H_2O$$

The water formed from the rapid quantitative reaction in the presence of pyridine between the amine and aldehyde is titrated with Karl Fischer reagent, after the excess aldehyde has been removed by means of the cyanhydrin reaction.[7]

The new procedure is applicable to aliphatic, alicyclic and aromatic primary amines and also amino alcohols which do not contain a secondary amino nitrogen group. Heterocyclic secondary amines interfere.

Combined with the acetylation technique for primary plus secondary amine,[8] the present method permits the estimation of secondary amine. When a total base titration is included, these procedures offer a means for determining rapidly and precisely the primary, secondary and tertiary amine content of mixtures.

Experimental

Analytical Procedure.—The sample, containing up to 0.1 equivalent of primary amine, is weighed into a 100-ml. volumetric flask about one-third filled with dry pyridine. After dilution to the mark with more pyridine, a 10-ml. portion is transferred to a 250-ml. glass-stoppered volumetric flask. Three ml. of benzaldehyde[9] is added, the flask stoppered and, together with a blank, placed in a 60° bath. After thirty minutes the flasks are removed and allowed to cool spontaneously to room temperature. The flasks are transferred to a well-ventilated hood. About 0.2 g. of dry sodium cyanide and 30 ml. of 6% hydrogen cyanide in pyridine are added. The flasks are shaken vigorously for about one minute[10] and then set aside in the hood for forty-five minutes. At the end of this time the mixture is titrated with Fischer reagent[11] to the usual visual end-point.[12]

The water found after correction for that present in the blank and original sample is equivalent to the amount of primary amine in the sample. Free water is best obtained by titrating the original sample in acetic acid solution.

Analytical Results

A group of seventeen widely different primary and primary–secondary amines was analyzed by the new method and reported in Table I. With the exception of *p*-bromoaniline, which was recrystallized from chloroform, the trade products were used without further purification. The precision and accuracy are usually within ±0.2%.

Results for *p*-bromoaniline were precise but consistently about 5% low as compared with the value obtained by the acetylation method. Urea and methyl urea reacted to the extent of about 10% on the basis of one mole of water formed per mole. This value would be doubled if the disubstitution product were formed.[13]

Amino alcohols containing only primary amine groups react quantitatively. This reaction, presumably, might follow one of two courses, either normal imine formation or condensation involving both the amine and hydroxyl to form a substituted oxazine type compound.[14] In this case the

(1) Presented in part before the Division of Analytical and Micro-Chemistry at the 106th meeting of the American Chemical Society, Pittsburgh, Pa.

(2) Yul'chevskaya, *et al.*, *Lab. Prakt.*, **16**, 6 (1941).

(3) Schoorl, *Pharm. Weekblad*, **77**, 1381 (1940).

(4) Ferry and Buck, THIS JOURNAL, **58**, 2444 (1936).

(5) Wanag, *Z. anal. Chem.*, **21**, (1938).

(6) Shupe, *J. Assoc. Off. Agr. Chem.*, **24**, 754 (1941).

(7) Bryant, Mitchell and Smith, THIS JOURNAL, **62**, 3504 (1940).

(8) Mitchell, Hawkins and Smith, *ibid.*, **66**, 782 (1944).

(9) Either freshly distilled or acid-free benzaldehyde inhibited with about 0.1% hydroquinone is preferred. Results with benzylamine were only 95% quantitative using benzaldehyde containing about 10% benzoic acid.

(10) This shaking is required to initiate the cyanhydrin reaction since the sodium cyanide catalyst is insoluble in the pyridine.

(11) Preferably in a well-ventilated hood since the mixture contains excess hydrogen cyanide.

(12) This titration should be fairly rapid. The first sharp end-point should be taken, for occasionally some fading may be observed after standing a few minutes.

(13) Schiff, *Ann.*, **291**, 368, 370, 371 (1896).

(14) Kohn, *Monatsh.*, **26**, 956 (1905), isolated 4,6,6-trimethyl-2-phenyltetrahydrometoxazine after treating benzaldehyde with 2-hydroxy-2-methylpentylamine 4.