portunity factor" by which extra time is given for degradation to occur, because of the time lag conferred by the need for bioconversion of the P(S) to P(O) (*10*). In this case, the selectivity is always such as to spare the mammal. Amiton is known to be degraded in liver (*14*). If this degradation was more vigorous in mammals than in insects, the thiono sulfur would have the effect of raising the mammalian LD_{50} by this same "opportunity factor," and so reducing adverse selectivity.

A paradox still unresolved is: if adverse selectivity in these compounds is due to failure of the protonated material to penetrate the nerve cord, why is (for example) the P(O)SFF still 13-fold selective, even though it has a pKa of 4.7, so that at pH 7 only 0.5% would be protonated? The effect is not attributable to poor lipid solubility of the free base, for its partition ceofficient, water: olive oil, was found to be 1.06. It could be caused by target selectivity—i.e., the cholinesterase of the housefly being more sensitive than that of the mouse. Alternatively, it may be connected with the fact that the housefly applications were topical, so that an extra barrier (the cuticle) was interposed in this case.

Certain compounds which were poor anticholinesterases in vitro were nevertheless toxic to flies or mice. In the case of P(S) compounds, the reason is undoubtedly that these are latent inhibitors, and are activated to P(O) derivatives in vivo. In one case, the P(O)OFF compound, toxicity to houseflies was encountered in spite of poor inhibition of fly cholinesterase in vitro (pI_{50}, 4.2), and no precedent exists for activation of such a compound to a more potent anticholinesterase. Suspicion that the fluorinated compounds might kill by some effect other than cholinesterase inhibition,—e.g., by forming fluoracetate from a fluoroethyl group—was lessened when it was found that one of the "half-molecules," (ethyl-2-fluorethyl)-2-aminoethanol, had little toxicity to mice, for 200 mg. per kg. (intraperitoneal) was not lethal.

Acknowledgment

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INSECTICIDES FROM PLANTS

Nicandrenone, A New Compound with Insecticidal Properties, Isolated from *Nicandra physalodes*

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An insect repellent substance with toxic properties has been isolated in chromatographically pure form from leaves of the plant *Nicandra physalodes*. The molecular formula $C_{34}H_{42}O_7$ has been established for the compound, and evidence is presented which indicates that it is a conjugated ketone. The name "nicandrenone" is suggested. Initial studies aimed at elucidation of the structure of the compound are reported; these include infrared, ultraviolet, and nuclear magnetic spectra.

IN NATURE, the tobacco hornworm feeds on only a few plants, all of which are members of the family *Solanaceae*. Laboratory feeding tests in which fresh foliage was used showed that many other plants of this family are palatable to the insect, although some which were readily eaten produced toxic effects (*13*). In the same experiment, a few solanaceous plants were found to be repellent with little or no feeding occurring on them. A plant in the latter category is *Nicandra physalodes*. The repellent factor can be extracted from the leaves of this plant with water and removed from the aqueous solution by extraction with chloroform or ether. Removal of solvent from the latter extracts leaves solid material which has been found to be highly toxic to house flies as well as to the hornworm (*2*). Fractionation of this crude material and investigations into the chemical composition of the purified toxic substance are reported in this study.

This material has been isolated by other investigators (*4*), but its repellent and toxic properties toward insects were not observed. The name "nicandrin" was proposed for what was probably an impure preparation. The conclusion was reached that nicandrin is a glycoside.

In searches for naturally occurring insecticides, previous investigations failed to demonstrate toxic activity in the plant *Nicandra physalodes* (*6*). Houseflies were used as test insects in one study in which petroleum ether, ethyl ether, and chloroform extracts of the whole plant were found to be nontoxic. Another investi-

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gation showed aqueous extracts of the whole plant and of the upper parts and fruits to be nontoxic to German and American cockroaches and to milkweed bugs (*6*).

Experimental Procedures and Results

Isolation of Crude Material. Crowns of the plant *Nicandra physalodes* were air-dried at room temperature. Leaves were than stripped from the stems and ground in a domestic corn mill. The resulting powder was stored in brown bottles at room temperature under which conditions it retained activity for as long as a year. For extraction of the active substance, 200 grams of leaf powder were suspended in 2 liters of hot tap water in a large beaker. This was allowed to stand in a boiling water bath for approximately 3 hours with frequent stirring of the contents. Solid material was then removed by suction filtration, and to it was added 1 liter of hot tap water for a second extraction. This time the mixture was allowed to stand at room temperature for approximately 18 hours with occasional stirring. After removal of the residue, the dark green filtrates from the two extractions were combined and saturated first with neutral then with basic lead acetate, the precipitated flavanoid substances being removed by suction filtration each time. This treatment prevented troublesome emulsions in extraction of the active material from the solution with chloroform. The pH of the solution was adjusted to 9.0 with ammonium hydroxide. Removal of the precipitate which formed at this step left an almost colorless filtrate whose volume was approximately 2 liters. This was divided into two equal parts, and each was extracted four times with 100-ml. portions of chloroform in a separatory funnel. The colorless chloroform extract was washed with distilled water, then dried over anhydrous sodium sulfate. The solvent was removed under vacuum at 45° to 55° C. A yellowing of the solution occurred as it became concentrated, and the solid residue after removal of all solvent was slightly yellowish. The yield of crude material obtained by this procedure was approximately 1.3 grams, and contained nine components as shown by the following chromatographic method.

Thin Layer Chromatography. Stahl's (*11*) thin layer chromatographic method (TLC) was used employing Desaga-Brinkmann equipment. Slides were prepared from Brinkmann Silica Gel G. Materials to be tested were spotted at the starting line in chloroform solution, and chromatograms were developed by the ascending method in closed jars (9.5 × 33.5 cm.) containing 50 ml. of 5% methanol in chloroform. The solvent front was allowed to reach a distance of approximately 12 cm. from the starting line, this process requiring about 1 hour. Slides were air-dried after the solvent front had been marked and were sprayed with a 10% solution of phosphomolybdic acid in methanol, a reagent which has been used in paper chromatography of steroids (*7*). Heating for approximately 5 minutes in an oven at 110° C. caused the appearance of dark blue or purple spots on a yellow background. These colors were stable for only a few days. R_f values varied considerably from one determination to another—e.g., a range of 0.820 to 0.689 for the fastest-moving component. Instead of trying to control all of the factors which might contribute to this variability, it was found more convenient to chromatograph a reference sample of crude material each time a fraction was tested.

Fractionation of the Crude Material. A slurry of Mallinkrodt silicic acid, 100 mesh, in chloroform was used to prepare a column 6.5 × 2.5 cm. A chloroform solution of 1.88 grams of the crude material was placed on this. Substances on the column were eluted first with chloroform, six 50-ml. cuts being collected. Following this, two 50-ml. fractions were taken using 5% methanol in chloroform for elution, then two using 10% methanol in chloroform. The Nicandra compounds described here give a bright, cherry-red color when treated with 85% phosphoric acid. Since this test is very sensitive, it was used for following the presence of material in fractions collected from columns. Solvent was removed from fractions under vacuum at 50° C., and the residues after being dried in a vacuum desiccator were weighed and chromatographed. Chromatograms showed that the substances were eluted in the order of their decreasing R_f values, each fraction containing from three to five components. Total recovery of material from the column was 1.759 grams or 93.5%, with most of it appearing in the first, second, seventh, and eighth cuts. Only the first two fractions, with residues weighing 886 and 162 mg., respectively, contained the compound with the highest R_f value, which was shown subsequently to be the biologically active material. Each of these fractions contained the first five compounds in the order of their decreasing R_f values on thin layer chromatograms.

Cut No. 1 was recrystallized from benzene–petroleum ether (b.p. 60°–65° C.) and the product from this treatment was shown by TLC to contain only two components: those having the first and third highest R_f values of all the compounds present in the crude material. These two compounds are called Nic-1 (higher R_f) and Nic-2 for convenience.

A chloroform solution of 845 mg. of such a mixture of Nic-1 and Nic-2 was placed on an 11- × 2.5-cm. silicic acid column prepared as described above. Again chloroform was used first for elution, three 50-ml. cuts being collected. The last drop from the third cut gave no color with 85% phosphoric acid, hence the solvent for elution was changed to 5% methanol in chloroform for one 50-ml. fraction then 10% methanol in chloroform for a final cut of the same volume. Solvent was removed from the fractions, and the residues were dried, weighed, and chromatographed. Cuts No. 1 and 2 yielded 261 and 259 mg. of material, respectively, and both fractions were shown to be chromatographically pure Nic-1. There was no residue from the third cut. The fourth yielded 314 mg. of material which was a mixture of Nic-1 and Nic-2. The residue from the final cut weighed only 8 mg. and was not chromatographed.

Cut No. 2 from the original column fractionation of the crude material was recrystallized from benzene. The product was shown by TLC to be pure Nic-2. No attempt was made to isolate other components of the crude material in pure form.

Toxicity Tests on Nic-1 and Nic-2. Female houseflies of the Wilson strain were used as test insects to determine toxicity of the two compounds. Solutions of the compounds were prepared by grinding a few milligrams of material with a drop of distilled water in an agate mortar then removing insoluble material by filtration through a sintered glass filter. Each fly received 0.425 μl. of the filtrate by injection. Control flies received the same volume of distilled water. Among 20 control flies and 26 flies receiving Nic-2 there were no mortalities, but all 25 flies receiving injections of Nic-1 died. The volume of solution of Nic-1 administered to each fly was shown to contain approximately 1 μg. of the compound.

When Nic-1 was added to natural or artificial diets for tomato hornworm larvae, the insects would not feed. When the compound was force-fed to them, they became paralyzed in a short time, and there was a high incidence of mortality.

Some Chemical and Physical Properties of Nic-1. Nic-1 is a bitter-tasting white compound of rather waxy consistency which melts at 102° to 105° C. It is optically active with $[\alpha]_D^{27} + 21.6°$ (c = 9, methanol). Under the usual conditions of solubility tests it is very sparingly soluble in water, as in 5% hydrochloric acid and 5% sodium hydroxide solution; but, on standing several hours in contact with the last solvent, it dissolves to a slight extent yielding a bright yellow solution. It is soluble in concentrated sulfuric acid giving a bright red color which quickly turns brown, and it dissolves in 85% phosphoric acid forming a cherry-red solution. The compound is soluble at room temperature in chloroform, ethyl ether, methanol, ethanol, acetone, ethyl acetate, and

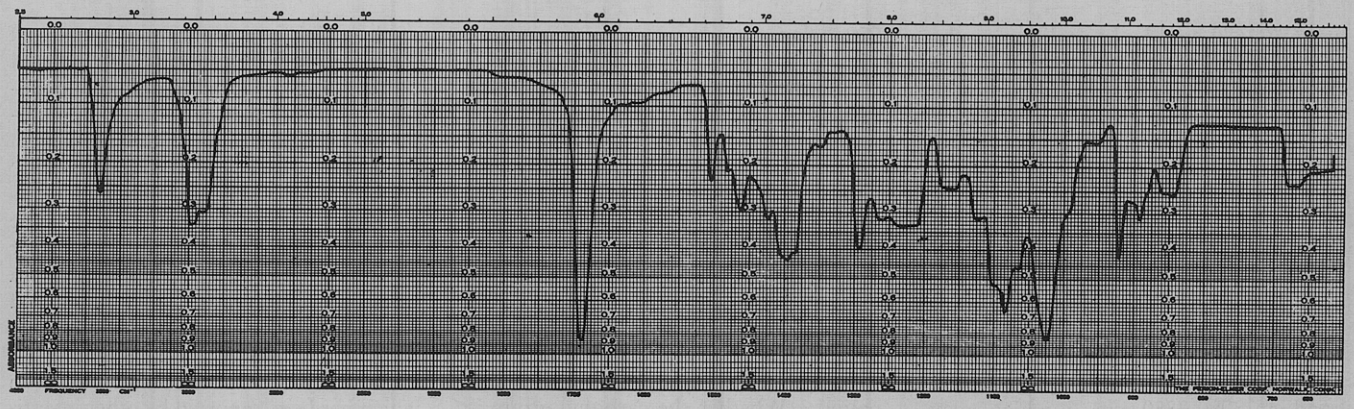

Figure 1. Infrared spectrum of Nic-1

solvent: $CHCl_3$; concentration: 10%; thickness: 0.1 mm.; prism: NaCl

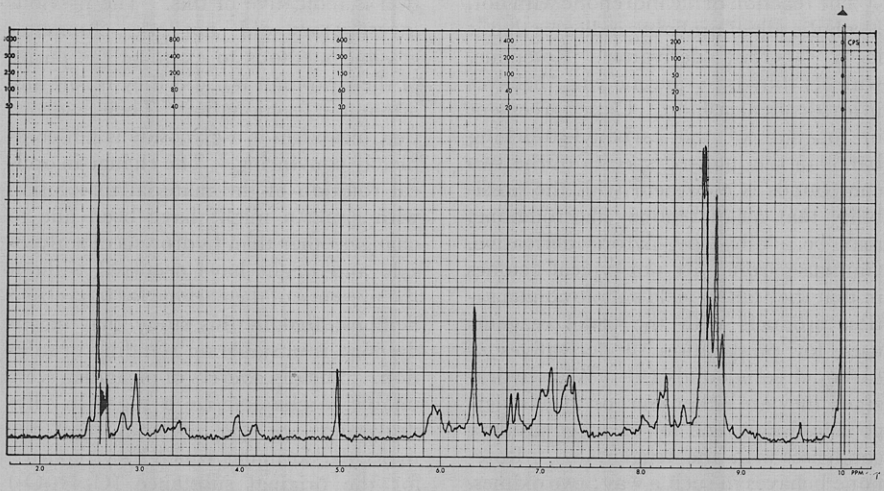

Figure 2. Nuclear magnetic resonance spectrum of Nic-1

Varan A-60 NMR spectrometer at 60 mc.; solvent: $CDCl_3$ with tetramethylsilane as an internal standard; concentration: 10%; sweep width: 500 c.p.s.

dioxane. Exact solubilities in these solvents have not been determined. It is soluble in hot benzene and crystallizes from this solvent.

Nic-1 has been shown to contain only carbon, hydrogen, and oxygen. Quantitative determinations for these elements added up to very close to 100%, and qualitative tests for halogen, nitrogen, and sulfur by standard methods were negative.

The 2,4-dinitrophenylhydrazine test on the compound is positive with the derivative being orange-colored. The Tollens' and Schiff's tests for aldehydes are negative; Nic-1 is therefore a ketone. Great difficulty was encountered in attempts to prepare the semicarbazone and thiosemicarbazone, and neither was ever obtained in pure form.

When a solution of the compound in alcoholic alkali is heated a dark orange-colored precipitate forms instantaneously. The naturally occurring insecticide rotenone behaves in an identical fashion under the same conditions.

Nic-1 does not give the colors typical of unsaturated lactones in the Légal test for such compounds. The compound exhibits the property of halochromism, giving brilliant colors with strong acids These solutions are decolorized on addition of water. Nic-1 in dry form is stable for long periods of time, but it is unstable in solution. A chloroform solution of the pure compound was allowed to stand in the refrigerator for 12 weeks, and then was examined by TLC. The heaviest spot was that of the original compound, but all of the spots appearing in the crude material were present plus three others of higher R_f value than that of Nic-1.

Percentages of carbon and hydrogen were determined on two samples of the chromatographically pure compound, both obtained from appropriate cuts from column fractionation but only one being subsequently crystallized from benzene. The compound retains traces of solvent tenaciously, particularly chloroform, as was shown by the fact that the sample which was not crystallized from benzene gave a positive Beilstein test for halogen after 5 hours' drying in an Abderhalden apparatus at 100° C. Its analytical values for carbon and hydrogen were in poor agreement with those of the sample that had been crystallized from benzene and dried in the same manner. However, after the chloroform sample was dried 15 hours longer under the same conditions, its analytical data were almost identical with those of the benzene sample. Since the compound contains only carbon, hydrogen, and oxygen, percentage of the last element in these samples was obtained by subtracting the sum of percentages of the other two from 100. Analytical data are as follows:

Chloroform sample: C, 72.63%; H, 7.45%; O, 19.92%.
Benzene sample: C, 72.64%; H, 7.42%; O, 19.94%.

The molecular formula calculated from these data is $C_{34.03}H_{41.50}O_7$. A compound having the formula $C_{34}H_{42}O_7$ has a molecular weight of 562.676 and the following analytical values: C, 72.57%; H, 7.52%; O, 19.91%. The molecular weight of Nic-1 determined in chloroform solution in a vapor pressure osmometer was 562. Thus, the data presented here are consistent for a molecular formula of $C_{34}H_{42}O_7$ for Nic-1.

There is evidence from the infrared spectrum (see next section) for the presence of the hydroxyl group in Nic-1. Quantitative determination of this group showed it to represent 2.95% of the compound. This indicates one hydroxyl group per molecule (actual calculated value, 0.98).

Determination of terminal methyl groups gave a value of 7.79% indicating that the compound contains three such groups per molecule (actual calculated value, 2.92).

Nic-1 contains no methoxyl groups.

Tests for hydroxyl, terminal methyl, and methoxyl groups were performed by standard methods (Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.).

Infrared Spectrum of Nic-1. The infrared spectrum of Nic-1 is shown in Figure 1. The occurrence of a very sharp, carbonyl-stretching band at a low frequency for this function (1693-1685 $cm.^{-1}$) suggests that the compound is a conjugated ketone (*1*). The infrared spectrum of rotenone measured in chloroform solution for comparison with that of Nic-1 showed a carbonyl-stretching band

at 1677-1670 cm.$^{-1}$ In addition to the C=O band, most ketones show a peak in the region 1350-1200 cm.$^{-1}$ (*1*). Nic-1 has a sharp peak at 1295-1290 cm.$^{-1}$ Carbonyl-stretching bands of most esters and lactones (saturated or unsaturated) occur at frequencies higher than 1700 cm.$^{-1}$ (*1*). Such bands are absent in the spectrum of Nic-1. The band at 3530-3520 cm.$^{-1}$ can be ascribed to the hydroxyl group present in Nic-1.

Carbon-hydrogen vibrations in HC=CH groups show up in the region 3040-3010 cm.$^{-1}$ (*1*). Nic-1 shows a broad band at 3000-2880 cm.$^{-1}$ which may represent this function. Aromatic compounds show this band near 3030 cm.$^{-1}$ and C=C bands near 1600 and 1500 cm.$^{-1}$ (*1*). Nic-1 has a sharp peak at 1505 cm.$^{-1}$, but none near 1600.

Ether linkages in large-membered rings probably absorb from 1150 to 1060 cm.$^{-1}$ (*1*). Nic-1 exhibits a band at 1085 cm.$^{-1}$ with shoulders at 1105 and 1065.

Ultraviolet Spectrum of Nic-1. The ultraviolet spectrum of Nic-1 in solution in 95% ethanol shows an intense absorption band at 217 mμ with ϵ = 17,670, and a broad band of low intensity from 290 to 370 mμ with $\lambda_{max.}$ at 333 mμ and ϵ = 167. Similar bands are typical of α, β-unsaturated ketones (*3*). In addition to these bands, the spectrum shows a group of sharp peaks with maximum wavelengths of 261, 267, 276, and 278 mμ and molar absorptivities of 326, 459, 416, and 273, respectively. These are suggestive of aromaticity in the compound (*3*).

The thiosemicarbazone of Nic-1 was prepared in very poor yield, and the product was of unknown purity so its ultraviolet spectrum can be considered only qualitatively. It showed peaks with $\lambda_{max.}$ at 243 and 296 mμ. The locations of these are in agreement with those of absorption bands found in spectra of thiosemicarbazones of α,β-unsaturated ketones (*3*).

Nuclear Magnetic Resonance Spectrum (NMR) of Nic-1. The NMR spectrum of the compound is shown in Figure 2. Lack of appreciable absorption above 8.9 τ (*12*) indicates that the methyl groups that are present are close to oxygen functions, double bonds, or aromatic rings (*5*). The peaks in the 8.5–8.9 τ region are due to methyls of the above-mentioned types and possibly to methylene groups. The lack of much absorption in the 8.0–8.5 τ region suggests that the compound is not a steroid (*10*) or triterpenoid derivative (*8*). The sharp peak at 5 τ is probably due to a proton attached to an alkene linkage (*5*). The peaks at 4 τ are due to a proton of the latter type. The absorption in the 2.4–3.5 region (4–6 protons) is due to aromatic protons, and possibly to some low double-bond protons (*5*).

Discussion

The molecular formula, $C_{34}H_{42}O_7$, of the insecticidal compound isolated from *Nicandra physalodes* is suggestive of a fused-ring structure. The calculated number of double bonds plus rings is 14. Some aromaticity in the molecule is indicated.

The infrared, ultraviolet, and nuclear magnetic resonance spectra all provide evidence for the presence of a conjugated keto group. For this reason, the name "nicandrenone" is proposed for the compound. Whether the carbonyl group is conjugated with an aromatic ring or with a single double-bond awaits elucidation.

The reaction of nicandrenone with hot, alcoholic alkali probably indicates that it is a γ-pyrone derivative. Rotenone, with its dihydro-γ-pyrone ring, behaves in an identical fashion. Close agreement in location of the carbonyl-stretching bands in the infrared spectra of the two compounds is consistent with the possibility that nicandrenone, like rotenone, may be a dihydro-γ-pyrone derivative. Gamma-pyrones are not typical ketones in that they fail to yield simple condensation products with the usual reagents used for preparation of derivatives of carbonyl compounds (*9*). With hydrazines, they are either inert or they undergo ring-fission, and the cleavage product condenses with the reagent (*9*). Rotenone behaves in such a way; two oximes, a hydrazone, and a phenylhydrazone have been prepared from this compound, and these are all thought to be derivatives of the product of opening of the chromanone (dihydro-γ-pyrone) ring (*9*). The presence of a similar ring in nicandrenone could account for its erratic behavior with carbonyl reagents (positive test with 2,4-dinitrophenylhydrazine but failure to condense easily with semicarbazide or thiosemicarbazide to yield pure derivatives).

Nicandrenone probably contains at least one aromatic ring. The ultraviolet and nuclear magnetic resonance spectra provide good evidence for aromaticity as does the peak at 1505 cm.$^{-1}$ in the infrared spectrum. Absence of a band near 1600 cm.$^{-1}$ in the last spectrum is not conclusive negative evidence because this band in aromatic compounds is known to be variable (*1*).

If nicandrenone is indeed a γ-pyrone derivative, this ring and the one hydroxyl group present account for three oxygen atoms in the molecule. The four that are not accounted for probably occur in ether linkage in the ring system. The band at 1085 cm.$^{-1}$ in the infrared spectrum is consistent with this possibility.

A previous paper reported that the repellent substance in *Nicandra physalodes* is probably a steroid (*2*). This conclusion was based largely on the fact that the material gives positive results in many of the color tests used for detection of steroids; these results can be explained by the property of halochromism exhibited by nicandrenone. That the compound probably is not a steroid is shown by the lack of absorption in the 8.0–8.5 τ region of the NMR spectrum and by its instability toward alcoholic alkali. The molecular weight reported in the earlier paper was determined on a preparation which was found later to be impure and was measured by the Rast method which is often unreliable.

Probably the substance nicandrin which has been reported by other investigators (*4*) represents a crude mixture of compounds such as the one described in this paper; similarity in method of isolation is indicative of this. The previous investigators did not use chromatographic methods to examine the purity of their product nor did they report any attempt at fractionation. Their evidence that nicandrin is a glycoside is not completely convincing. The change in optical rotation of the material in solution with emulsin might have been due to changes other than hydrolysis of a glycoside and might have occurred without the presence of the enzyme for it has been demonstrated in this study that nicandrenone is unstable on standing in solution. The discoverers of nicandrin were unable to characterize a sugar in "hydrolysates" of the material, and the molecular formulas which they reported for the original substance ($C_{27}H_{37}O_7$) and the "aglycone" ($C_{20}H_{26}O_5$) do not suggest the splitting-off of a sugar moiety. That nicandrenone, on the other hand, is not a glycoside is evident from the fact that it contains only one hydroxyl group per molecule.

Acknowledgment

Infrared, ultraviolet, and nuclear magnetic resonance spectra were measured in spectroscopic laboratories of the Chemistry Department of the University of Illinois. Carbon and hydrogen percentages and molecular weight were determined in the microanalytical laboratory of the Chemistry Department of the University of Illinois. The authors thank R. B. Bates of the Chemistry Department of the University of Illinois for interpretation of the NMR spectrum and S. C. Chang, U.S.D.A., A.R.C., Entomology Research Division, Beltsville, Md., for toxicity tests on houseflies. This investigation was supported in part by grants from the Rockefeller Foundation, N. Y., and the Graduate College of the University of Illinois.

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FRUIT THINNER RESIDUES

Residue Determination of Naphthaleneacetic Acid in Olives

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A method for the determination of small residues of naphthaleneacetic acid in olives, which had been treated with 150 p.p.m. of this plant growth regulator shortly after bloom, has been developed. The chloroform extract of raw olives is purified by chromatography on alumina and silica gel, followed by esterification with diazomethane and separation of the methyl ester by gas-liquid chromatography. Final analysis is by spectrophotometry at 360 mμ with an over-all sensitivity of 0.1 p.p.m. Residues of naphthaleneacetic acid ranged during one season's treatment from an initial concentration of 0.5 p.p.m. to nondetectable at harvest time, 142 days later. Comparable results from the 1962 season ranged from 1.6 p.p.m. initially to nondetectable amounts at harvest time, 125 days later.

THE USE of naphthaleneacetic acid (NAA) as a fruit thinner for olives (*5, 6*) has prompted the development of a sensitive analytical method for residue determinations of this plant growth hormone. A recently developed method for NAA employs the nitration of the naphthalene ring and the subsequent development of a characteristic color (*1*). An adaptation of this method for the determination of NAA on olives proved unsuccessful due to high blanks, and a rigorous cleanup technique had to be devised, based on column and gas-liquid chromatography followed by nitration and spectrophotometry. The cleanup step involving gas liquid chromatography was an adaptation of the residue method for methyl ester naphthaleneacetic acid (MENA) in potatoes (*7*).

Experimental

Apparatus and Equipment. Gas liquid chromatograph Aerograph A-90-C, equipped with 6-foot, 1/4-in. o.d. copper column packed with 20% w./w. silicone high vacuum grease Dow 11 on acid-washed Chromosorb P. Heated exit port is modified with 7/15 standard taper stainless steel inner joint.

Fraction collectors are 1.0 × 14 cm. glass tubes, tapered at one end and fitted with a 7/15 standard taper outer joint at the other. A small piece of absorbent cotton is inserted into the collector and saturated with chloroform just prior to collecting (*8*).

Spectrophotometer, Beckman Model DU; cylindrical silica cells, 10 mm. o.d., 100 mm. light path (Pyrocell Mfg. Co., New York 28, N. Y.).

Reagents. DIAZALD-STABILIZED DIAZOMETHANE [*N*-methyl-*p*-tolylsulfonylnitrosamide (Aldrich Chemical Co.)]. Diazomethane is freshly generated once every 2 weeks by the following method. Seven grams of Diazald is dissolved in about 5 ml. of anhydrous diethyl ether and placed in a dropping funnel attached to a distillation flask. Ten grams of potassium hydroxide dissolved in 8 ml. of water and 20 ml. of 95% ethanol are transferred into the distillation flask kept at 0° C. The temperature is slowly raised to 70° C. in a water bath, and the Diazald solution is added dropwise. With continuous agitation provided by a magnetic stirrer, the ethereal solution of diazomethane is distilled into a 25-ml. volumetric receiving flask cooled in ice. Additional ethyl ether is slowly added through the dropping funnel until about 25 ml. of distillate has been collected. The diazomethane solution may be stored for several weeks in a desiccator at −10° C. Since diazomethane is toxic and explosive, only rubber connections are used on the apparatus, and all operations are carried out in a well-ventilated hood.

ALUMINA, basic, grade 1 (Woelm).

SILICIC ACID (Mallinckrodt 2844); activated at 160° C. for 4 hours and stored in a desiccator.

1-NAPHTHALENEACETIC ACID (Distillation Products).

NAA Calibration. One milliliter of a standard solution of NAA (1.00 mg. per ml. of chloroform) was esterified by the addition of 1.0 ml. of a freshly prepared diazomethane solution in ether. After the cessation of gas evolution, indicating the completion of the reaction, the solution was concentrated to 1.0 ml. in a graduated centrifuge tube with a stream of air.

Aliquot volumes of this solution ranging from 0 to 25 μl. were injected into a gas chromatography column by means of a 100-μl. Hamilton syringe. Operating conditions of gas chromatography were: 210° C. column temperature, 50 ml. per min. helium gas flow. The retention time for MENA as detected by gas conductivity katharometer was between 4 to 6 minutes. Fractions were collected manually at this predetermined retention time by attaching the glass collector to the heated exit port (*7*).

The condensed MENA was washed from the collector into a 50-ml. Erlenmeyer flask with 15 ml. of chloroform and evaporated to dryness with warm

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