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THE STRUCTURE OF AFFININ, THE INSECTICIDAL AMIDE FROM *ERIGERON AFFINIS* D. C.

FRED ACREE, JR., MARTIN JACOBSON, AND H. L. HALLER

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The isolation of an insecticidally active amide, affinin, from the roots of *Erigeron affinis* D. C. has been described in a previous article (1).

As obtained on distillation of the purified nitromethane-soluble constituents separated from the petroleum ether extractives of the roots, affinin is a thick oil with a marked tendency to polymerize. From its chemical behavior, including the production of succinic acid on permanganate oxidation, and from spectrographic data it was concluded that the compound is most likely N-isobutyl-2,6,8-decatrienoamide. Since the yield of succinic acid was low and the nature of the volatile acids had not been determined, there was the possibility that the amide might consist of a constant-boiling mixture of the 2,6,8 and the 2,5,7 isomers.

More recent evidence has indicated that affinin is a homogeneous compound. When it was crystallized from acetone solution with the aid of solid carbon dioxide, the separated crystals, after melting below room temperature, had a refractive index essentially identical with that of the original substance. Further permanganate oxidations of affinin have resulted in the isolation of acetic, isobutyric, oxalic, and N-isobutyloxamic acids, in addition to succinic acid. While the yields of oxidation products are lower than normally might be expected, this is probably due to polymerization. The absence of propionic acid in the volatile acid mixture, together with the crystallization data presented above, is considered to be proof that affinin is N-isobutyl-2,6,8-decatrienoamide and contains none of the 2,5,7 isomer.

An attempt to obtain more nearly quantitative yields of degradation products by the ozonization of affinin was unsuccessful, acetaldehyde being the only reaction product that was isolated and identified.

EXPERIMENTAL

Crystallization of affinin. A solution of 300 mg. of affinin dissolved in 2 ml. of acetone was allowed to crystallize, and the crystals were separated by centrifugation. Both operations were performed while the centrifuge tube was cooled in a mixture of solid carbon dioxide and acetone. The separated crystals melted below room temperature, and after the last traces of solvent were removed under reduced pressure, the remaining oil had essentially the same refractive index (n_D^{27} 1.5120) as that of the original affinin (n_D^{27} 1.5128). The only similar situation found thus far in the literature occurs in the case of the isomeric 1,3- and 2,4-hexadienes, whose refractive indices are n_D^{12} 1.4416 and n_D^{22} 1.4490, respectively (Prevost 2).

Partial permanganate oxidation of affinin. Finely ground potassium permanganate (36.4 g., equivalent to 6 moles of oxygen) was added in small portions to a continuously stirred suspension of 6.35 g. of affinin in 500 ml. of water maintained at 40–50°. When the reaction mixture had become colorless, the manganese dioxide was filtered and washed thoroughly, first with warm water, then with acetone, and finally with ether. The acetone and ether filtrates, when combined and dried, yielded 0.67 g. of viscous residue on removal of the solvent. The residue appeared to be polymerized material, and it was not examined further.

After having been evaporated to a small volume, the combined aqueous filtrates were acidified with sulfuric acid, steam-distilled to remove the volatile acids (36.08 ml. *N* NaOH), and then extracted completely with ether. The solvent was removed from the ether solution, and the 2.63 g. of partly crystalline residue after two recrystallizations from ethyl acetate yielded 520 mg. of substance, m.p. 185–186°.

Anal. Cal'd for $C_4H_6O_4$: C, 40.65; H, 5.12; mol. wt. 118.

Found: C, 40.93, 40.97; H, 5.12, 5.08; mol. wt. by titration, 119.

The substance was identified as succinic acid by a mixture melting point determination, m.p. 185–186°. An additional 400 mg. of succinic acid (total yield 27%) was obtained from the ethyl acetate mother liquors, together with an oily residue which could not be made to crystallize further. The residue gave a positive test for nitrogen and it decomposed on distillation. [In another experiment 6.63 g. of affinin was oxidized over a period of 2 weeks, in a manner similar to that just described, with a large excess of permanganate (76.4 g., equivalent to 12 moles of oxygen). However, it was not possible to isolate more than 800 mg. of succinic acid or 24% of the theory.]

The neutral solution of steam-volatile acids was evaporated until crystals appeared. The mixture was cooled and acidified with an excess of sulfuric acid and then filtered through a bed of charcoal to remove a small quantity of precipitate. On repeated steam distillation by the Dyer procedure as modified by Hillig and Knudsen (3), the filtrate was found to be a mixture of two relatively volatile acids which were identified as isobutyric acid (45 mg.) and acetic acid (1.28 g. or 0.74 mole). In addition a less volatile acid was isolated by extracting the steam-distillation residues with ether. The ether solution was dried, and the solvent was removed, yielding 1.32 g. of crystalline material, which was digested with 200 ml. of boiling ligroin (b.p. 58–70°). The hot solution was filtered from some insoluble material and concentrated to one-half volume by boiling. After being cooled, the solution deposited 0.43 g. of substance, m.p. 106–107° (sublimation 80–90°), which contained nitrogen, and an additional 100 mg. of the substance was obtained on removal of the solvent from the ligroin mother liquor. A considerable loss of material apparently occurred from boiling the ligroin solutions in open beakers. The combined portions of crystalline substance sublimed completely at 105–110°, $p = 15$ mm., and melted at 107°.

Anal. Calc'd for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.66; mol. wt., 145.

Found: C, 49.80, 50.28; H, 7.55, 7.57; N, 9.82, 9.87; mol. wt. by titration, 146.

The substance was identified as N-isobutyloxamic acid by a mixture melting point determination with an authentic sample, m.p. 107° (*Anal.* Found: mol. wt. by titration, 146) which was prepared by the reaction of 1 mole of ethyl oxalate on 1 mole of isobutylamine according to the procedure of Malbot (4) and which sublimed at 105–110°, $p = 15$ mm.

The charcoal residue was dried and combined with the ligroin-insoluble residue and then completely extracted with anhydrous ether. On removal of the solvent the ether solution yielded 300 mg. of substance, from which 75 mg. of unidentified oily material was separated by sublimation at 105–125°, $p = 15$ mm. When the pressure was reduced to 0.3 mm., 215 mg. of crystalline material was obtained which sublimed at 93–96°. The sublimate was intimately mixed with 5 ml. of chloroform and then filtered. After the solvent was removed, the filtrate yielded an additional 100 mg. of practically pure N-isobutyloxamic acid (*Anal.* Found: C, 49.56, 49.69; H, 7.29, 7.36).

The chloroform-insoluble residue was dried and sublimed, yielding 100 mg. of substance melting at 186–187°, which contained no nitrogen and rapidly reduced an aqueous solution of potassium permanganate.

Anal. Calc'd for $C_2H_2O_4$: C, 26.7; H, 2.24.

Found: C, 26.3, 25.9; H, 2.33, 2.30.

This substance was identified as anhydrous oxalic acid by a mixture melting point determination.

Ozonization of affinin. An excess of ozone was bubbled through a cold solution of 1.6 g. of affinin dissolved in 20 ml. of dry carbon tetrachloride. The ozonide precipitated before the reaction was completed. After the solvent was removed at 20°, p = 15 mm., 15 ml. of water was added, and the ozonide was decomposed while the bath temperature was slowly increased to 75° over a period of 2 hours. During this time the volatile aldehydes were swept from the flask with a stream of nitrogen and were absorbed in a cold solution of 2 g. of dimethyldihydroresorcinol dissolved in 600 ml. of water. The solution was then stoppered tightly, left for 24 hours at about 40–50°, and finally cooled overnight. The crystalline material which separated was filtered, dried, and weighed; yield 0.43 g. It was identified as ethylidenedimethone by its sharp melting point, 139°, which is the melting point recorded in the literature (Vorländer 5) for this compound. From the yield and the solubility of the substance it was calculated that 24% of the theoretical quantity of acetaldehyde was recovered. No other readily volatile aldehydes appeared to have been produced.

The aqueous mixture containing the nonvolatile aldehydes was made alkaline and 10 ml. of 30% hydrogen peroxide was added in small portions, with shaking, until frothing ceased. A small amount of insoluble gummy material was dissolved by the addition of a few milliliters of acetone, and the reaction mixture was concentrated to half its volume by boiling. The small quantity of precipitate that separated was filtered from the hot solution, which was then cooled and acidified and filtered again to remove an additional small quantity of insoluble material. The filtrate was continuously extracted with ether, the solvent was removed from the ether solution, and the residue was steam-distilled. Distillation curves obtained on treatment of the distillate (8.55 ml. *N* NaOH) by the Dyer procedure (Hillig and Knudsen, 3) indicated that it consisted of a mixture of formic, N-isobutyloxamic, and acetic acids. The presence of the first two acids was further substantiated when the distillate gave a qualitative test for nitrogen and also readily reduced a solution of mercuric chloride. The formic acid was found to have come from the oxidation of the acetone, and the acetic acid was no doubt formed during the decomposition of the ozonide.

The liquid residue from the first steam distillation was filtered from a small quantity of gummy material and evaporated to dryness, yielding 0.9 g. of residue. A portion of this residue, which partly crystallized, readily reduced a solution of mercuric chloride and therefore contained some formic acid. No other substance could be identified in the residue.

SUMMARY

Affinin has been found to be a pure substance. The nature of the oxidation products has led to the conclusion that the compound is N-isobutyl-2,6,8-decatrienoamide.

BELTSVILLE, MD.

REFERENCES

(1) ACREE, JACOBSON, AND HALLER, *J. Org. Chem.*, **10**, 236 (1945).
(2) PREVOST, *Ann. chim.* **10**, 147–181 (1928).
(3) HILLIG AND KNUDSEN, *J. Assoc. Official Agr. Chem.* **25**, 176 (1942).
(4) MALBOT, *Compt. rend.* **104**, 229 (1887).
(5) VORLÄNDER, *Z. anal. Chem.*, **77**, 326 (1929).