[CONTRIBUTION FROM THE ENTOMOLOGY RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE]

Constituents of *Heliopsis* Species. III.[1] *cis-trans* Isomerism in Affinin

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Treatment of affinin, the insecticidal amide from the roots of *Heliopsis longipes*, with ultraviolet light or with selenium yields all-*trans*-affinin, which is no longer toxic to house flies. Proof of structure is shown by hydrogenation to N-isobutylcapramide and by permanganate oxidation to acetic, oxalic, succinic and N-isobutyloxamic acids. Chemical and spectral data throw light on the stereochemical configuration of the natural isomer and confirm the *trans* configuration of its conversion product.

The isolation of affinin, an insecticidal amide, from the roots of the Mexican plant submitted as *Erigeron affinis* DC. and its identification as N-isobutyl-2,6,8-decatrienamide (I), were reported in 1945 by Acree, *et al.*[2] In 1947 the plant was shown to be *Heliopsis longipes* (A. Gray) Blake[3,4] (family

$$CH_3CH{=}CHCH{=}CH(CH_2)_2CH{=}CHC(=O)NHCH_2CH(CH_3)_2$$

I

compositae), and a detailed report of its insecticidal activity was published by McGovran, *et al.*[5]

There are eight possible *cis* and *trans* isomers having structure I. Although the geometrical configuration of affinin was not definitely determined, its failure to yield a maleic anhydride adduct suggested that at least one of the two conjugated double bonds possesses the *cis* configuration.[6]

Previous work with unsaturated isobutylamides has shown that *cis–trans* isomerism plays an important part in their physiological and insecticidal activity. For example: N-isobutyl-*trans*-2-*trans*-4-decadienamide caused marked paralysis of mucous membranes and was about half as toxic as pyrethrins to house flies, while the same compound with at least one double bond (probably both) possessing the *cis* configuration was comparatively inactive.[7,8] On the other hand, N-isobutyl-*trans*-2-*trans*-4-dodecadienamide is only weakly pungent and is completely inactive toward house flies.[9] It was therefore of interest to determine the configuration of affinin and to prepare and evaluate the corresponding all-*trans* compound.

The natural affinin used in the present investigation was isolated by the procedure previously reported,[2] from roots of the same batch that had been stored in the dark at room temperature for 6 years. Although roots extracted when first received had yielded about 1% of affinin, the yield after 6 years was only 0.4% (dry-root basis). The pale-yellow oil obtained boiled at 157° at 0.26 mm., $n^{25}D$ 1.5134, and crystallized in the cold as colorless needles, m.p. 23°.

Exposure of a solution of affinin, in dry petroleum ether containing a trace of iodine, to ultraviolet light for 2.5 hours gave 50% of a compound, $C_{14}H_{23}NO$, crystallizing as colorless needles, m.p. 91.5°. Quantitative hydrogenation to N-isobutylcapramide, m.p. 38–38.5°, and oxidation to N-isobutyloxamic, acetic, oxalic and succinic acids showed it to be isomeric with affinin. The same compound could be obtained in 25% yield by heating affinin with selenium at 225° for 2 hours. Inasmuch as treatment of a *cis* compound with ultraviolet light[10] or with selenium[11] has been shown to cause elaidinization to the *trans* isomer, the new compound was designated "*trans*-affinin." It readily gave an adduct, m.p. 175°, with maleic anhydride.

The geometrical relationship between affinin and *trans*-affinin is borne out by their ultraviolet absorption spectra (Fig. 1) and infrared spectra (Fig. 2).[12] The ultraviolet spectra are practically identical, both compounds exhibiting the maximum at 228.5 mμ. However, the ε value for affinin is 33,700, while that for *trans*-affinin is 37,100. It has been shown[13] that *cis* and *trans* isomers usually exhibit almost identical maxima, but that the *trans* compound shows a considerably higher intensity. Jackson, *et al.*,[14] have found that in the infrared the conjugated *trans,trans*-methyl linoleates are characterized by a single strong band at 988 cm.$^{-1}$ and the *cis,trans* isomers by two bands of medium intensity at 948 and 982 cm.$^{-1}$. Evidence along the same lines also has been found by Celmer and Solomons[6] in the case of mycomycin, and by Paschke, *et al.*[15] This behavior is shown definitely in the case of affinin (943 and 976 cm.$^{-1}$) and *trans*-affinin (985 cm.$^{-1}$ only), and is additional evidence

(1) Part I, M. Jacobson, THIS JOURNAL, **73**, 100 (1951); part II, **74**, 3423 (1952).

(2) F. Acree, Jr., M. Jacobson and H. L. Haller, *J. Org. Chem.*, **10**, 236 (1945); **10**, 449 (1945).

(3) (a) M. Jacobson, F. Acree, Jr., and H. L. Haller, *ibid.*, **12**, 731 (1947); (b) E. L. Little, *J. Wash. Acad. Sci.*, **38**, 269 (1948).

(4) Although it was proposed (ref. 3a) that the use of the name "affinin" for natural I be discontinued, this designation is used throughout the present paper for purposes of convenience.

(5) E. R. McGovran, *et al.*, U. S. Bur. Ent. and Plant Quar. E-736, 5 pp. (1947).

(6) The Diels–Alder reaction invariably fails or proceeds very poorly when the conjugated diene contains a *cis* bond. See W. D. Celmer and I. A. Solomons, THIS JOURNAL, **75**, 1372 (1953), and other references cited therein.

(7) L. Crombie, *Chemistry and Industry*, 1034 (1952).

(8) M. Jacobson, THIS JOURNAL, **75**, 2584 (1953).

(9) M. Jacobson, unpublished report.

(10) (a) R. C. Calderwood and F. D. Gunstone, *Chemistry and Industry*, 436 (1953); (b) U. Eisner, *et al.*, *J. Chem. Soc.*, 1372 (1953); (c) S. C. Gupta, *et al.*, *J. Sci. Ind. Res. (India)*, **10B**, 76 (1951); (d) P. L. Nichols, *et al.*, THIS JOURNAL, **73**, 247 (1951).

(11) D. Swern, *et al.*, *J. Am. Oil Chemists' Soc.*, **27**, 17 (1950); J. H. Skellon and J. W. Spence, *Chemistry and Industry*, 302 (1953).

(12) The infrared spectra were determined by Jonas Carol, of the U. S. Food and Drug Administration. The bands at 4.40 and 4.75 mμ are due to absorption by the carbon disulfide in which the spectra were prepared.

(13) See Crombie, ref. 7; Eisner, ref. 10b; H. P. Koch, *Chemistry and Industry*, **61**, 273 (1942).

(14) J. E. Jackson, *et al.*, *J. Am. Oil Chemists' Soc.*, **29**, 229 (1952).

(15) R. F. Paschke, W. Tolberg and D. H. Wheeler, *ibid.*, **30**, 97 (1953).

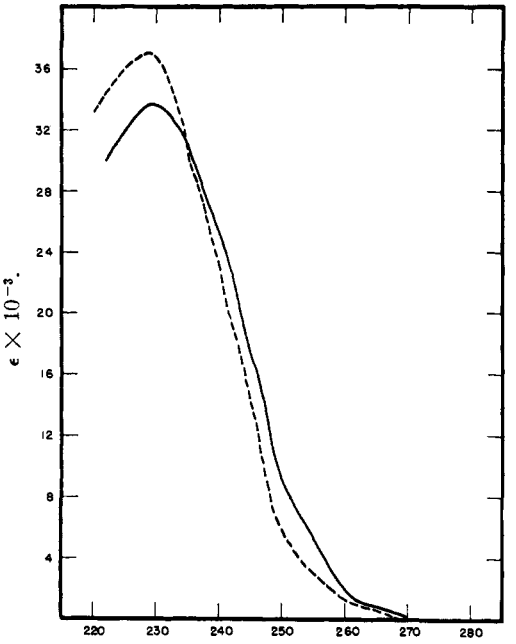

Fig. 1.—Ultraviolet spectra of affinin (solid line) and *trans*-affinin (broken line) in 95% alcohol; concentration 2×10^{-6} mole per liter.

for a *cis* configuration at the C-6 or C-8 double bond in affinin. The band at 818 cm.$^{-1}$ in affinin is almost completely absent in the case of *trans*-affinin. This is in line with Crombie's data[16] for the geometrical isomers of N-isobutyl-2,8-dodecadienamide, showing that a band at this point is markedly reinforced as one goes from *trans,trans* to *cis,cis*, and may indicate that the α,β-double bond in affinin possesses a *cis* configuration (an unusual feature in a naturally occurring acid derivative). The same is true for the band at 725 cm.$^{-1}$ in affinin.

In contrast to affinin, which is highly pungent and has the same order of paralyzing action and toxicity to house flies as the pyrethrins, *trans*-affinin is only weakly pungent and non-toxic to house flies, but it does exhibit a high paralytic action on this insect.[17] Both isomers show approximately the same degree of instability at room temperature in the presence of air and light, turning to a dark-colored resin after several weeks. However, they may be preserved at 5° for at least a year without deterioration.

Experimental[18]

Affinin.—The natural isomer was isolated in 0.4% yield (based on dry root) from *Heliopsis longipes* roots stored in the dark at room temperature for 6 years, by the procedure previously reported.[2] It was obtained as a pale-yellow, viscous oil, b.p. 157° (0.26 mm.), 141° (0.2 mm.), m.p. 23°, $n^{25}D$ 1.5134, $\lambda_{max.}$ 228.5 mμ in 95% ethanol (ε 33,700).

***trans*-Affinin.**—Affinin (5 g.) was dissolved in 30 ml. of Skellysolve B, a small crystal of iodine was added, and the solution was exposed to the direct light from an ultraviolet lamp for 2.5 hours while being cooled with an electric fan to prevent evaporation. By the end of this period the mixture had crystallized to a solid waxy mass. It was melted by warming, 70 ml. of Skellysolve B was added, and the mixture was heated to boiling on the steam-bath. The clear yellow solution was separated from an insoluble thick orange oil by decantation, boiled with 1 g. of charcoal, filtered, and concentrated to 30 ml. on the steam-bath. On cooling, a mass of white crystals separated, which was filtered off and washed with a little cold petroleum ether. Four recrystallizations from this solvent gave 2.5 g. (50%) colorless needles, m.p. 91.5°. The compound could be distilled in an atmosphere of nitrogen without decomposition; b.p. 145° (0.25 mm.), $\lambda_{max.}$ 228.5 mμ in 95% ethanol (ε 37,100).

Anal. Calcd. for $C_{14}H_{23}NO$: C, 76.08; H, 10.49; N, 6.32. Found: C, 76.14; H, 10.40; N, 6.27.

The orange oil (2.2 g.) insoluble in boiling petroleum ether proved to be an intractable resin soluble only in hot pyridine.

The crystalline compound, m.p. 91.5°, could also be obtained by heating 1.5 g. of affinin with 12 mg. of selenium at 225° for 2 hours, cooling, adding 20 ml. of ether, filtering, and removing the solvent on the steam-bath. Recrystallization of the crystalline residue from petroleum ether gave 375 mg. (25%) of long, colorless needles, m.p. 91.5°, and no depression on admixture with the compound described above.

The compound (200 mg.), heated at 100° with 200 mg. of maleic anhydride for 1 minute and then cooled, gave a crystalline *adduct;* colorless needles (from benzene), m.p. 175°.

Anal. Calcd. for $C_{18}H_{25}NO_4$: C, 67.68; H, 7.89; N, 4.38. Found: C, 67.54; H, 7.91; N, 4.42.

Hydrogenation of *trans*-Affinin.—An ethanol solution containing 0.5490 g. of *trans*-affinin was hydrogenated with 50 mg. of reduced platinum oxide catalyst. In 15 minutes 158 ml. (cor.) of hydrogen was taken up, and the reaction abruptly ceased. (The theoretical requirement for 3 moles of hydrogen for this weight of a substance of molecular weight 223 is 158 ml.) The reaction mixture was separated from the catalyst, and the solvent was removed at reduced pressure, leaving 550 mg. of a colorless oil which crystallized in rosettes of needles, m.p. 38–38.5°, identical with a specimen of N-isobutylcapramide, m.p. and mixed m.p. 38–38.5°.

Oxidation of *trans*-Affinin.—To a stirred suspension of 2 g. of *trans*-affinin in 120 ml. of water, maintained at 75°, 12 g. of finely powdered potassium permanganate (equivalent to 6 moles of oxygen) was added in small portions. When the reaction mixture had become colorless, the manganese dioxide was filtered and washed thoroughly with warm water. The combined aqueous filtrates were concentrated down to 35 ml. and made acid to congo red with sulfuric acid. The solution was steam-distilled to remove the volatile acids and then extracted with ether in a continuous extractor. The ether solution was freed of solvent, and the partly crystalline residue was subjected to sublimation in a micro-sublimator. Five hundred and two mg. of colorless solid was obtained, which sublimed at 100–105° (15 mm.) and melted at 185–186° dec. It reduced rapidly an aqueous solution of potassium permanganate, and was identified as anhydrous oxalic acid (62%) by a mixed melting point de-

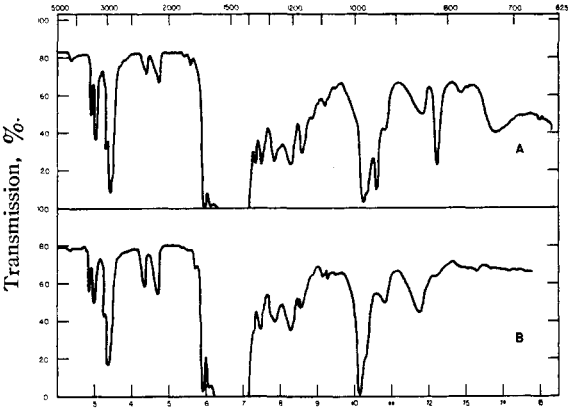

Fig. 2.—Infrared spectra in carbon disulfide: A, affinin, B, *trans*-affinin.

(16) L. Crombie, *J. Chem. Soc.*, 2997 (1952).

(17) The tests against house flies were made by W. A. Gersdorff and N. Mitlin.

(18) All melting points are corrected; boiling points are uncorrected.

termination with an authentic specimen (m.p. 186–187° dec.).

The sublimation residue, after recrystallization from ethyl acetate, yielded 582 mg. (55%) of colorless crystals, m.p. 187–188°, identified as succinic acid by a mixed melting point determination with an authentic specimen (m.p. 187.5–188.0°).

The solution of steam-volatile acids obtained above was neutralized with sodium hydroxide solution, concentrated to a small volume on the steam-bath, and acidified to congo red with sulfuric acid. The solution was rapidly steam distilled, the distillate neutralized with 0.1 *N* sodium hydroxide solution, and the neutral solution evaporated to dryness and the *p*-phenylphenacyl ester prepared. It melted at 111° and a mixed melting point determination with authentic *p*-phenylphenacyl acetate, m.p. 111°, showed no depression.

The distillation residue was extracted with ether in a continuous extractor, and the ether solution was dried and freed of solvent. The crystalline residue sublimed completely at 90–95° (15 mm.). Recrystallization from Skellysolve B gave 845 mg. (65%) of colorless feathery needles, m.p. 107°, identified as *N*-isobutyloxamic acid by a mixed melting point determination with a synthetic sample, m.p. 107°.

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[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1a]]

The Structure of α-Amylase Modified Waxy-Corn Starch[1b]

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Graded hydrolysis of waxy-corn starch by malt α-amylase produced a series of modified amylopectins which were characterized by their reducing power, the amount of formic acid produced on periodate oxidation and their convertibility by β-amylase. From these determinations, the average chain length and the average inner- and outer-branch lengths were calculated. These calculated values decreased as the extent of hydrolysis progressed. Hence, the modified products are best represented as polymers differing from the parent amylopectin in molecular weight and in the average length of both inner and outer branches. It is concluded further that the initial hydrolysis by malt α-amylase is not confined to the outer branches, but that the longer inner branches are attacked simultaneously. Similar studies on modified β-limit dextrins confirmed this conclusion.

It is known that when branched starch substances are hydrolyzed by malt α-amylase, the 1,4-glucosidic linkages are broken and the 1,6-glucosidic linkages at branch points are stable.[2,3] Our most precise knowledge of the mode of attack by the various α-amylases comes from studies of the low-molecular-weight products liberated from the branched substrates in the early[4] or in the final[3] stages of hydrolysis. There appear to have been few investigations of the residual polysaccharide produced during the early stages of hydrolysis.[5] Myrbäck[6] has contended that malt α-amylase hydrolyzes most rapidly those 1,4-linkages near the middle of the linear portions of the molecule and that long chains are more susceptible to attack than short chains. That is, the 1,4-linkages in the neighborhood of branch points (or end groups) are cleaved at a lower rate than those farther away. His picture is based almost wholly on data obtained from studies of the sugars and oligosaccharides that are split away from the polysaccharide substrate. Kinetic studies by Schwimmer[7] support the concept of dependence of rate of hydrolysis on the number of anhydroglucose units in a chain, provided that the chain is composed of less than about ten units. It is evident that assessment of the structure of the modified amylopectin remaining after slight hydrolysis would serve to test Myrbäck's theory.

We have treated waxy-corn starch with graded amounts of malt α-amylase to produce a series of modified amylopectins. These modified amylopectins were precipitated by addition of alcohol to 60% concentration and characterized by their reducing power, by the amount of formic acid produced from them on periodate oxidation and by their convertibility by β-amylase (Table I).

TABLE I

PROPERTIES OF MODIFIED WAXY-CORN STARCHES

α-Amylase units/g.	Yield, %	Reducing power[a]	Non-reducing end group, %	β-Convertibility, %
0	..	..	4.8	55
1.5	81	13.7	6.2	55
2.5	78	17.4	6.7	52
5.0	65	33.8	8.5	50
10.0	62	38.8	9.2	44

[a] Maltose hydrate equivalent, mg./g.

The percentage non-reducing end group and the average chain length (the number of anhydroglucose units per non-reducing end group) were calculated from the formic acid production. Some of our materials have appreciable reducing power and it is necessary to correct the formic acid values for the two moles of acid arising from each mole of reducing end group.[8] The proportion of anhydroglucose units in the outer branches is given by the "β-amylase convertibility"—the fraction of the molecule removed as maltose on treatment with β-amylase. The product of convertibility and average chain length then gives the average number of anhydroglucose units removed from the outer branches. To obtain the average outer-branch length, there must be added to this product a constant value to allow for the "stubs," at branch points, that β-amylase is not able to digest. Follow-

(1) (a) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture, article not copyrighted. (b) Presented before the Division of Carbohydrate Chemistry at the 125th National Meeting of the American Chemical Society, Kansas City, Mo., March, 1954.

(2) K. Myrbäck, *Advances in Carbohydrate Chem.*, **3**, 251 (1948).

(3) K. H. Meyer and W. F. Gonon, *Helv. Chim. Acta*, **34**, 308 (1951).

(4) R. Bird and R. H. Hopkins, *Biochem. J.*, **56**, 86 (1954).

(5) K. Myrbäck and R. Lundén, *Arkiv. Kemi Mineral. Geol.*, **23A**, No. 7, 1 (1946).

(6) K. Myrbäck and L. G. Sillén, *Svensk Kem. Tidskr.*, **56**, 60 (1944); K. Myrbäck, *Arch. Biochem.*, **14**, 53 (1947).

(7) S. Schwimmer, *J. Biol. Chem.*, **186**, 181 (1950).

(8) K. H. Meyer and P. Rathgeb, *Helv. Chim. Acta*, **31**, 1545 (1948); A. L. Potter and W. Z. Hassid, THIS JOURNAL, **70**, 3488 (1948).