[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND BOTANY, UNIVERSITY OF CALIFORNIA, LOS ANGELES]

Xanthinin: a Plant Growth-regulating Compound from *Xanthium pennsylvanicum*. I

BY T. A. GEISSMAN, PETER DEUEL, ERIK K. BONDE AND F. A. ADDICOTT

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A crystalline compound, xanthinin, has been isolated from the leaves of *Xanthium pennsylvanicum* (Compositae). Xanthinin is an unsaturated keto lactone with the composition $C_{17}H_{22}O_5$, and can be converted by the loss of a molecule of acetic acid into the previously reported xanthatin, $C_{15}H_{18}O_3$. Xanthinin and xanthatin appear to be related to the lactones already known in other *Compositae:* helenalin, tenulin and the santonins.

The recognition of auxin antagonism in extracts of the leaves of *Xanthium pennsylvanicum* (cocklebur)[1] led to studies in these laboratories directed to the isolation and chemical examination of the active principle. A crystalline compound is readily isolated from the young leaves of the plant, in which it appears to be present in considerable quantity (more than 1% of the dry weight). This substance, to which the name "xanthinin" has been given, is a colorless, crystalline compound with the composition $C_{17}H_{22}O_5$. It is a neutral compound, possessing a carbonyl group, a lactone grouping, an acetyloxy group, and at least one carbon–carbon double bond.

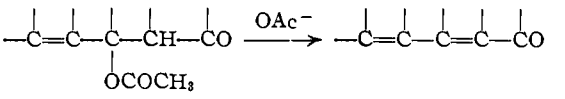
In the preliminary examination of the compound an attempt was made to acetylate it by treating it with acetic anhydride and sodium acetate. The product of this reaction was a compound which is not an acetate of xanthinin, but appears to be derived from xanthinin by the removal of the elements of acetic acid. Its composition is $C_{15}H_{18}O_3$; and whereas xanthinin shows no characteristic absorption in the ultraviolet above 220 mμ (Fig. 1a), the new compound shows an intense, well-defined absorption maximum at 275 mμ (log ϵ 4.30) (Fig. 1b).

Desacetylxanthinin appears to be identical with the compound "xanthatin" isolated from the same plant by Little, Foote and Johnstone[2] in their examination of the cocklebur for its antibacterial principle. Since the method of isolation used by these workers involved a chromatographic separation, with the use of alumina, xanthinin was probably converted to the desacetyl compound on the column. That xanthinin can be transformed to xanthatin in this way was demonstrated in the present study: a benzene solution of xanthinin was applied to an alumina column and after development with benzene and ether no xanthinin was found, but xanthatin was isolated from the ether eluate. The composition reported[2] for xanthatin was $C_{14}H_{16}O_3$; our analytical results are in better agreement with the formulation

$$\underset{\text{xanthinin}}{C_{17}H_{22}O_5} \longrightarrow \underset{\text{xanthatin}}{C_{15}H_{18}O_3} + CH_3COOH$$

Both xanthinin and xanthatin are lactones: the first consumes two equivalents of alkali, one for the lactone function, the second for the acetyloxy group; xanthatin consumes one equivalent of alkali. Both compounds give strongly positive hydroxamic acid tests, and neither gives a Legal reaction or reduces Tollens reagent. Xanthinin and xanthatin are optically active.

The lack of characteristic absorption in the ultraviolet by xanthinin, and the intense absorption at 275 mμ by xanthatin suggest the structural relationship between the two. The absorption of xanthatin is characteristic of an $\alpha,\beta,\gamma,\delta$-doubly-unsaturated carbonyl compound. Since xanthatin arises from xanthinin by the loss of acetic acid, the newly-formed double-bond could be formed in the manner shown in the partial formulation



That the acetic anhydride that was used in the first preparation of xanthatin from xanthinin played no essential part in the reaction was shown by the conversion of xanthinin into xanthatin by the action of sodium acetate in alcohol or acetic acid solution.

When xanthinin was saponified with alcoholic alkali and the acidified solution distilled with steam, one equivalent of volatile acid, identified as acetic acid, was obtained. Under these conditions xanthatin was not formed; either the action of alkali is to saponify the acetyloxy grouping with the formation of the corresponding hydroxy compound, or the xanthatin that may have resulted from the initial elimination of acetic acid was further modified by the alkali. That xanthatin does not survive treatment with alkali is indicated by the fact that no xanthatin could be recovered after the determination of its saponification equivalent. Xanthatin appears to be stable in neutral alcoholic solution when kept in the dark, as shown by the persistence of the high-intensity ultraviolet absorption maximum at 275 mμ. The compound is unstable when its solution is allowed to stand in the light, the peak absorption at 275 mμ disappearing completely in about two weeks.

The infrared absorption spectra of xanthinin and xanthatin, shown in Figs. 1 and 2, support the foregoing structural interpretations, and afford additional information. Xanthatin (Fig. 2) shows a sharp and well-defined absorption maximum at 1760 cm.$^{-1}$, characteristic of a saturated 5-membered lactone.[3] The sharp peaks at 1670 and 1600 cm.$^{-1}$ undoubtedly represent the $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl system, and a comparison with model systems[4] indicates that the 1670 cm.$^{-1}$ band represents the carbonyl group and the 1600 cm.$^{-1}$ band the carbon–carbon double bond(s). The remainder of the spectrum discloses the absence of a

(1) E. K. Bonde, *Physiol. Plantarum*, **6**, 232 (1953).

(2) J. E. Little, M. W. Foote and D. B. Johnstone, *Arch. Biochem.*, **27**, 247 (1950).

(3) J. F. Grove and H. A. Willis, *J. Chem. Soc.*, 877 (1951).

(4) R. N. Jones, V. Z. Williams, M. J. Whalen and K. Dobriner, THIS JOURNAL, **70**, 2024 (1948).

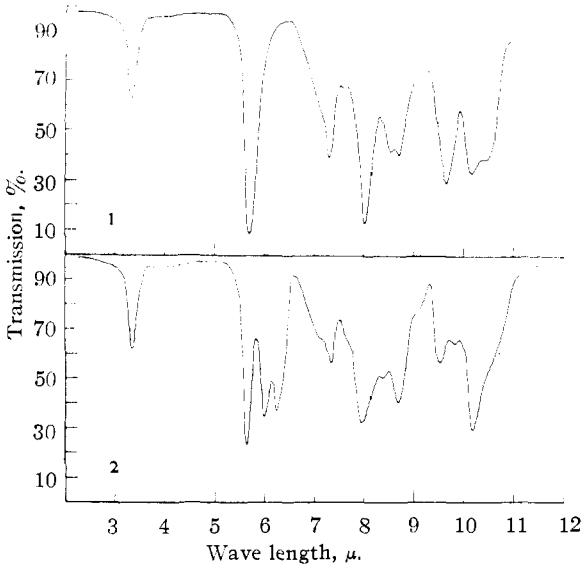


Fig. 1.—Infrared absorption spectrum of xanthinin; solvent, chloroform.

Fig. 2.—Infrared absorption spectrum of xanthatin; solvent, chloroform.

hydroxyl group, only the —C(OH)—H band at 3000 cm.$^{-1}$ appearing the 3-μ region. A strong absorption peak at 1150 cm.$^{-1}$ is probably due to a methyl group or groups, and bands at 1362 cm.$^{-1}$ (1370 in xanthinin) and about 1400 cm.$^{-1}$ (inflections) in both xanthatin and xanthinin correspond with the splitting in this region characteristic of the isopropyl group.[5] Xanthinin shows a single, broader peak at 1750 cm.$^{-1}$, readily accounted for as a combination of the three peaks arising from the lactone, saturated carbonyl and ester grouping known to be present in the molecule. No clear evidence of a band near 6 μ for the olefinic double bond appears but the width of the main band is such that moderate absorption in this region might be present. Xanthinin shows no hydroxyl absorption and, as mentioned above, shows absorption suggestive of the isopropyl grouping. Xanthinin shows sharp absorption at 958 and 984 cm.$^{-1}$, while xanthatin retains only the latter peak (983 cm.$^{-1}$). Although 958 cm.$^{-1}$ is slightly low, this band may represent the *trans*-CH=CH— grouping, an interpretation supported by the appearance of a sharp, low-intensity peak at 1316 cm.$^{-1}$.[6] The *trans*-CH=CH— grouping characteristically shows absorption at about 965–970 cm.$^{-1}$ (strong) and about 1300 cm.$^{-1}$ (weak). The absence of absorption at 900–910 cm.$^{-1}$ shows that the compounds do not contain the >C=CH_2 grouping.

Nothing in the ultraviolet or infrared spectra suggests the involvement of the lactone moiety in the dienone system. Neither xanthinin nor xanthatin (Fig. 3) shows more than end absorption (almost perfectly linear on the wave length–optical density plot) down to 210 mμ, ruling out the possibility that the lactone is α,β-unsaturated (in which case an absorption maximum in the region 210–215 mμ would have been observed).

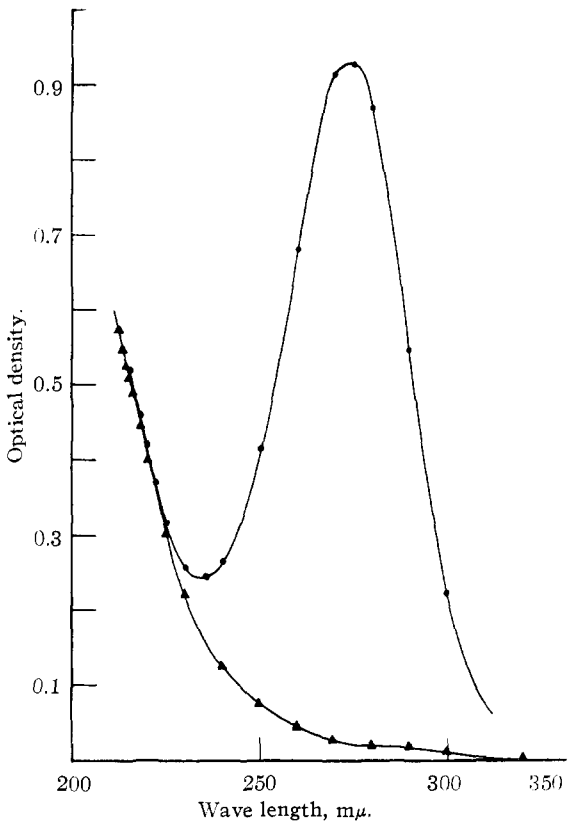


Fig. 3.—Ultraviolet absorption spectra of xanthinin and xanthatin; solvent, 95% ethanol: ●–●, xanthatin; ▲–▲, xanthinin.

The spectra of xanthatin show so remarkable a similarity to those of patulin, patulin acetate and patulyl chloride[7] as to suggest that very similar systems are present in xanthatin and patulin. In xanthatin, however, it is the keto and not the lactone carbonyl group that is a part of the 275 mμ chromophore, since the 2,4-dinitrophenylhydrazones of xanthinin and xanthatin are, respectively, golden-yellow and garnet-red in color, and show absorption maxima at 358 mμ and 389 mμ, respectively. Braude and Jones[8] have described the ultraviolet absorption spectra of the 2,4-dinitrophenylhydrazones of saturated and unsaturated carbonyl compounds and report that saturated ketone-DNPH show maxima at 362–365 mμ and α,β-γ,δ-unsaturated ketone-DNPH at 379–395 mμ.

In the present work satisfactory analyses for xanthinin and xanthatin-DNPH could not be obtained, but the *p*-bromophenylhydrazone of xanthinin yielded analytical figures in good agreement with the formula $C_{17}H_{22}O_5$. Attempts to prepare oximes were not completely successful, and the semicarbazones of xanthinin and xanthatin proved to be identical, and had an absorption spectrum

(5) N. Sheppard and D. S. Simpson, *Quart. Rev.*, **7**, 19 (1953).

(6) N. Sheppard and D. S. Simpson, *ibid.*, **6**, 1 (1952).

(7) H. J. Dauben and F. L. Seisenborn, THIS JOURNAL, **71**, 3853 (1949). The values of λ_{max} (ultraviolet) and the pertinent infrared bands for patulin and its derivatives are 275, 277, 277 mμ; 5.58, 5.58, 5.61; 5.94, 5.93, 5.94; and 6.11, 6.11, 6.13.

(8) E. A. Braude and E. R. H. Jones, *J. Chem. Soc.*, 498 (1945).

which showed that under the conditions used to prepare the derivative the xanthinin → xanthatin conversion took place.

The compositions of xanthinin and xanthatin are reminiscent of those of tenulin[9] ($C_{17}H_{22}O_5$), helenalin[10] ($C_{15}H_{18}O_4$), and the members of the santonin group (santonin,[11] $C_{15}H_{18}O_3$). Although the structures of tenulin and helenalin are still unknown, a close interrelationship of this group of compounds is strongly suggested by the observations that they are all lactones, the C_{17}-compounds tenulin and xanthinin both contain acetyloxy groups, and they are all found in the plant family *Compositae*.

Experimental

Isolation of Xanthinin.—Fresh leaves of young plants of *Xanthium pennsylvanicum* were collected, air-dried, and ground in a Wiley mill. The dry weight represented 21% of the fresh cut leaves. Fifty grams of leaf-powder was soaked in 800 ml. of acetone at room temperature, with frequent shaking, for 2 hours, then filtered under suction and the filtrate evaporated to dryness on a steam-bath. Five liters of distilled water was added to the dry residue and after 24 hours the water was filtered off and extracted with 3 l. of ethyl ether in 3 portions. The combined ether extracts were put into a deep-freezer to freeze out the water. The ether was filtered off and reduced to 15-ml. volume.

Xanthinin precipitated from the ether solution on cooling. The crystals were filtered off, washed with cold ether and twice recrystallized from the minimum amount of dilute alcohol. From each sample of 50 g. of leaf-powder was obtained 0.5–0.6 g. of xanthinin; this represents 1–1.2% of the dry weight of leaves or 0.21–0.25% of the weight of fresh leaves.

Physical and Chemical Properties of Xanthinin.—Xanthinin crystallizes in colorless plates from ether, alcohol or dilute alcohol; m.p. 121–122°. The compound has no odor. It burns cleanly, leaving no residue after ignition. Sodium fusion showed the absence of nitrogen, sulfur and halogens. It is practically insoluble in cold water, more soluble in hot water, very soluble in pyridine and soluble in acetone, alcohol and benzene. It is slightly soluble in ether and insoluble in petroleum ether. A neutral compound, it dissolves in concentrated sulfuric acid to give an orange solution, in concentrated hydrochloric acid with a deep purple color, in 85% phosphoric acid with light yellow color. In hot 5% sodium hydroxide a yellow solution is formed.

Xanthinin decolorizes bromine in chloroform; it gives a brown precipitate with cold permanganate solution, a precipitate with 2,4-dinitrophenylhydrazine, no mirror with Tollens reagent, no color with Schiff reagent, a red precipitate with Fehling solution on heating, no color with ferric chloride, and a purple color in the hydroxamic acid test for esters.

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.45; H, 7.36; OCH_3, none; rotation $[\alpha]^{25}D$ −53.0° (*c* 0.119 g. in 5 ml. of $CHCl_3$).

2,4-Dinitrophenylhydrazone of Xanthinin.—To a solution of 50 mg. of xanthinin in 3 ml. of 95% ethyl alcohol was added a hot solution of 75 mg. of 2,4-dinitrophenylhydrazine in 10 ml. of ethanol. After the addition of a drop of concentrated hydrochloric acid and brief boiling a golden-yellow precipitate appeared. The solution was cooled, the derivative collected, washed with alcohol, and recrystallized twice from chloroform–methanol mixture, m.p. 229–230°.

Anal. Calcd. for $C_{23}H_{26}O_8N_4$: C, 56.78; H, 5.39. Found: C, 56.01; H, 5.19.

Xanthinin *p*-Bromophenylhydrazone.—A solution of 250 mg. of xanthinin and 300 mg. of recrystallized *p*-bromophenylhydrazine in 10 ml. of alcohol was heated under reflux for 30 minutes. The solution was filtered and concentrated somewhat: on cooling the hydrazone crystallized. Recrystallized from alcohol, the compound formed yellow crystals, m.p. 138–138.5°.

Anal. Calcd. for $C_{23}H_{27}O_4N_2Br$: C, 58.11; H, 5.72. Found: C, 57.96; H, 5.88.

Semicarbazones from Xanthinin and Xanthatin.—A solution of 200 mg. of xanthatin, 300 mg. of semicarbazide hydrochloride and 0.5 ml. of pyridine in 5 ml. of absolute ethanol was refluxed for one hour. The residue obtained by evaporation of the solvents was washed repeatedly with water. It could not be satisfactorily recrystallized and had no definite melting point, decomposing over a range of temperature.

Anal. Calcd. for $C_{16}H_{21}O_3N_3$: C, 63.34; H, 6.98. Found: C, 62.82; H, 6.96.

When xanthinin was treated in the same way the same product was formed: both derivatives had identical absorption spectra (λ_{max} 290 mμ, log ε 4.69); from the position and intensity of the absorption maximum the derivative appears to possess the conjugated system of xanthatin.

Saponification of Xanthinin.—44.3 mg. (0.145 mmole) of xanthinin was dissolved in 3 ml. of ethyl alcohol, 4.30 ml. of 0.097 *N* sodium hydroxide was added, and the mixture warmed at 75° for 20 minutes, and back-titrated to the phenolphthalein end-point with 1.10 ml. of 0.113 *N* hydrochloric acid; milliequivalents of base consumed 2.93, calcd. for two saponifiable groups 2.90.

Identification of Acetic Acid.—A saponified solution of xanthinin was acidified with sulfuric acid. The solution was distilled and the distillate was collected, made slightly basic with dilute sodium hydroxide solution, and evaporated to dryness. To 0.200 g. of the dry sodium salt dissolved in 2 ml. of water, 0.200 g. of *p*-bromophenacyl bromide and 2 ml. of ethyl alcohol were added and the solution was made slightly acid with dilute hydrochloric acid. The mixture was refluxed for 1 hour on the steam-bath. *p*-Bromophenacyl acetate crystallized on cooling. Recrystallized from dilute alcohol, it had m.p. 82–83° (reported[12] 85°).

Anal. Calcd. for $C_{10}H_9O_3Br$: C, 46.72; H, 3.5. Found: C, 47.05; H, 3.38.

Desacetylxanthinin (Xanthatin). Method I.—Fifty milligrams of xanthinin was refluxed for one hour with several times its weight of sodium acetate in ethyl alcohol, and the solution cooled and diluted with water to cloudiness. The crystals that formed were filtered and recrystallized from dilute alcohol; m.p. 111–112°.

Anal. Calcd. for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 73.33; H, 7.06.

Method II.—A solution of xanthinin in benzene was passed through a column of alumina (which was not reactivated before use). The benzene filtrate contained neither xanthinin nor xanthatin. Ether was then passed through the column. Xanthatin, but no xanthinin, could be isolated from the ether eluate. The xanthatin was identified by its melting point, ultraviolet spectrum, and microanalysis.

Anal. Calcd. for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 72.99; H, 7.21; rotation $[\alpha]^{20}D$ −20.0° (*c* 0.122 g. in 5 ml. of $CHCl_3$).

2,4-Dinitrophenylhydrazone of Xanthatin.—The same procedure was used as in the case of xanthinin; garnet-red crystals, m.p. around 240° with decomposition.

Anal. Calcd. for $C_{21}H_{22}O_6N_4$: C, 59.15; H, 5.20. Found: C, 58.30; H, 5.23.

Saponification of Xanthatin.—40.3 mg. (0.164 mmole) of xanthatin was dissolved in 3 ml. of ethyl alcohol, 3.0 ml. of 0.097 *N* sodium hydroxide was added, and the solution warmed below the boiling point for 20 minutes. A back titration to the phenolphthalein end-point with 0.113 *N* hydrochloric acid required 1.03 ml.; calcd. for one equivalent of alkali per mole of xanthatin, 1.12 ml. 0.113 *N* acid.

LOS ANGELES, CALIFORNIA

(9) H. E. Ungnade and E. C. Hendley, THIS JOURNAL, **70**, 3921 (1948).

(10) R. Adams and W. Herz, *ibid.*, **71**, 2546 (1949).

(11) G. R. Clemo and R. D. Haworth, *ibid.*, **52**, 2579 (1930).

(12) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 222.