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THE CHEMISTRY OF A STRUCTURALLY DIAGNOSTIC COLOR REACTION OF XANTHININ AND RELATED SESQUITERPENE LACTONES*†

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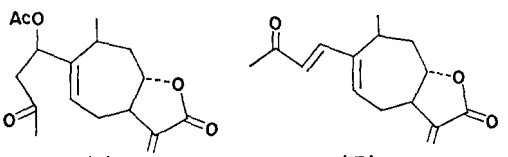
Abstract-Certain sesquiterpene lactones, among them xanthinin (1) and cumambrin-B (17), develop striking colors when treated with mineral acids. The structure of the colored compound derived from (1) and (17) is that of a tetraenylic cation, deprotonation of which gives rise to a fulvene derivative. The color-producing reaction is that of an acid-catalysed dimerization of an intermediate guaianolide. The structural requirements for the reaction are such that it serves as a useful guide in structure elucidation.

INTRODUCTION

IT HAS been known for many years by botanists working with the cockleburr, *Xanthium strumarium* L., that extracts of the plant develop pink to magenta colors when heated with mineral acids.[1] Later studies showed that the color-producing constituent of the plant is xanthinin (1)[2–5] or its C-8 epimer, xanthumin (2).[6] The corresponding dienone, xanthatin (3) and the 11,13-dihydro compounds also give the same color, showing that the 11,13-double bond of (1), (2) and (3) is not involved in the reaction.



(1)
(2, lactone, *c/s*)

(3)

Continuing studies of sesquiterpene lactones derived from various Composit ae species have revealed that the color reaction is not unique to xanthinin and its immediate allies, but is shown by numerous (but not all) compounds of the class. For this reason, and because the color reaction has been found to have usefully diagnostic structural limitations, a thorough study has been made of the phenomenon with the aim of establishing the nature of the reaction and the structure of the colored species.

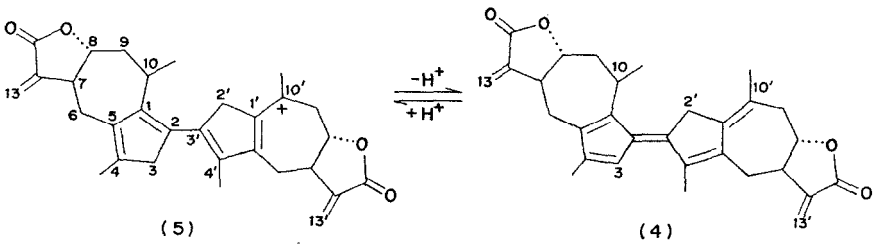
* Contribution No. 2408 from the Department of Chemistry, U.C.L.A.
† Taken in part from the Ph.D. Dissertation of T. S. Griffin, 1970.

[1] K. C. HAMNER. **Private communication.**
[2] J. E. LITTLE, M. W. FOOTE and D. B. JOHNSTONE, Arch. *Biochem. 27,247 (1950).*
[3] T. A. GEISSMAN, P. G. DEUEL, E. K. BONDE and F. A. ADDICOTT, *J. Am. Chem. Soc. 76, 685 (1954).*
[4] P. D. DEUEL and T. A. GEISSMAN, *J. Am. Chem. Soc. 79, 3778 (1957).*
[5] T. A. GEISSMAN, *J. Org. Chem. 27, 2692 (1962).*
[6] E. WINTERS, T. A. GEISSMAN and D. SAFIR, *J. Org. Chem. 34,* 153 (1969).

RESULTS AND DISCUSSION

The typical color produced in the reaction to be described here is a deep burgundy red with an absorption maximum at about 540 nm.* The color is clearly due to an ionic species for it is quite insoluble in ether and upon neutralization (deprotonation) is converted into a yellow, ether-extractable compound. Acidification of the yellow ether solution regenerates the red protonated species.

The behavior of xanthinin (1) is typical, and was selected for detailed study. The conditions for producing the colored species are not critical: gentle warming in strong (6-12 N) aqueous HCI, or addition of HCI to an alcoholic solution of xanthinin, followed by warming, is sufficient. Other strong acids (trifluoroacetic, sulfuric, boron trifluoride, perchloric, formic, and others) produce visually identical results. When the red solution in hydrochloric acid was neutralized, there was formed an orange compound which was obtained in crystalline form, and which redissolved in acid with regeneration of the red solution. This compound is assigned the structure (4) and is derived by loss of a proton from the red cation (5).† The evidence leading to these formulations is given in the discussion to follow.



(5) (4)

The fulvene (4) crystallizes as orange prisms which decompose and show no melting point when heated slowly, but which melt at 235-240" when introduced into a preheated bath. It gives a single spot on TLC.‡,§ The composition of (4) was found by elemental analysis to be $C_{15}H_{16}O_2$, and since the molecular ion was observed to have m/e 456, the compound has the composition $C_{30}H_{32}O_4$. The IR spectrum of (4) shows only a single strong peak in the carbonyl region at 1760 cm- ', indicating that γ-lactone rings are intact. A band at 1567 cm-', which disappeared upon hydrogenation, can be assigned to the carbon-carbon double bonds.

The absorption spectrum of the fulvene in chloroform shows maxima at 388 nm (ϵ 3 x 10^5) and 248 nm (ϵ 8·6 x 10"). The red cation (5) present in the solution of (4) in hydrochloric acid has λ_{max} 541 nm ($\epsilon = 10^5$). Spectra of (5) in sulfuric, formic and trifluoroacetic acids show maxima very near to this value (Table 1).

The 541 nm absorption maximum for (5) is in close agreement with the maxima that have been observed for other aliphatic polyenylic cations containing four conjugated double bonds. These include the symmetrical cation (6) generated from the polyene (7) by direct

* A second kind of color reaction shown by some lactones of this class is a deep blue, with an absorption maximum of about 595 nm. This reaction, and a survey of all of the compounds showing the red and blue reactions, will be described in a separate article (T. S. Griffin and T. A. Geissman, *Phytochem.* **10,** 2475 ***(1971).***

† The cation is represented for convenience by the single formal structure (5). The positive charge is of course delocalized over the system shown.

‡ A yellow spot which becomes red when sprayed with sulfuric acid.

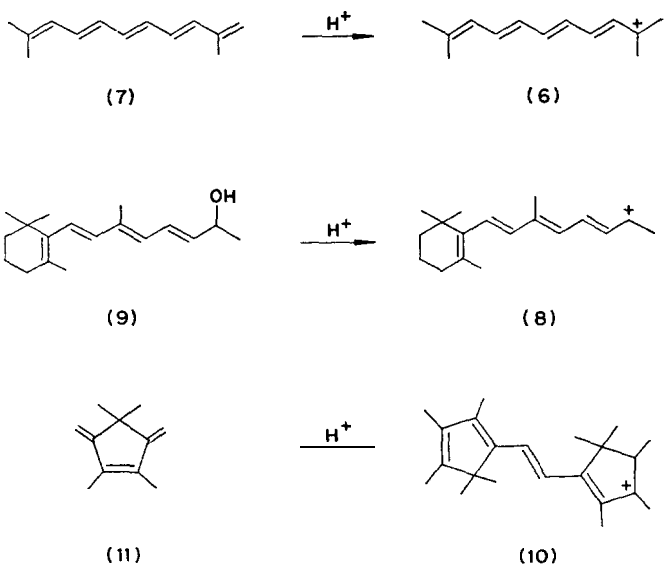
§ Analysis by TLC of the orange solution containing crude (4) before crystallization shows two additional spots at lower R_f which also turn red upon being sprayed with sulfuric acid. These may be the compounds with one or both of the lactone rings opened.

TABLE 1. ABSORPTION MAXIMA OF TETRAENYLIC CATIONS

Compound	Acid	λ_{max}(nm)	$\epsilon \times 10^{-4}$	Ref.
(5)	Conc. HCl	541	10	—
	80 % H_2SO_4	540	12	—
	CF_3COOH	535	11	—
	HCOOH	537		
(6)	80 % H_2SO_4	550	15	7
	Heptafluorobutyric	536	8	
(8)	Sulfuric/n-butanol	539	2.5	8
(10)	CH_3SO_3H/CH_3COOH	552	4.9	9

protonation;[7] cation (8) derived from the tetraenol (9)[8] and the cation (10) generated by acid-catalysed dimerization of the triene (11).[9]

Cations containing five conjugated double bonds are blue and show absorption maxima in the range 594-625 nm,[7,10] while those with but three double bonds are yellow and show maxima at 452-472 nm.[7,10] It is clear that the characteristics of the red cation are in accord with its formulation as (5).



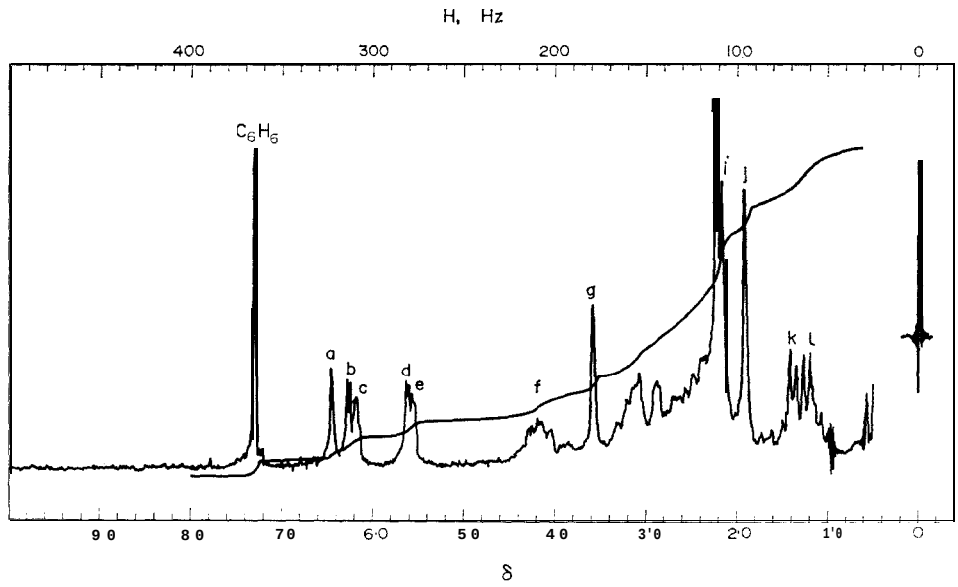
(7) (6)

(9) (8)

(11) (10)

Evidence to substantiate the formulations (5) and (4) for the cation and its progenitor is found in the NMR and mass spectra of the compounds. The NMR spectra of (4) and (5) are shown in Figs. 1 and 2, and show the following features. In the NMR spectrum of the fulvene (4) (Fig. 1) are seen two 3-proton doublets (each J = 7 Hz), at δ1·38 and 1·24, which are assigned to the secondary methyl groups at C-10 of (4). Two configurations are possible

[7] T. S. SORENSON, *J. Am. Chem. Soc. 87, 5075 (1965).*
[8] P. E. BLATZ, D. L. PIPPERT and V. BALASUBRAMANIYAN, *Photochem.PhotobioZ.8,* 309 (1968).
[9] L. DE VRIES, *J. Am. Chem. Soc.* 83, 2392 (1961).
[10] P.E. BLATZ, D. L. PIPPERT, L. SHERMAN and V. BALASUBRAMANIYAN, *J. Chem. Ed.* 46, 512 (1969).

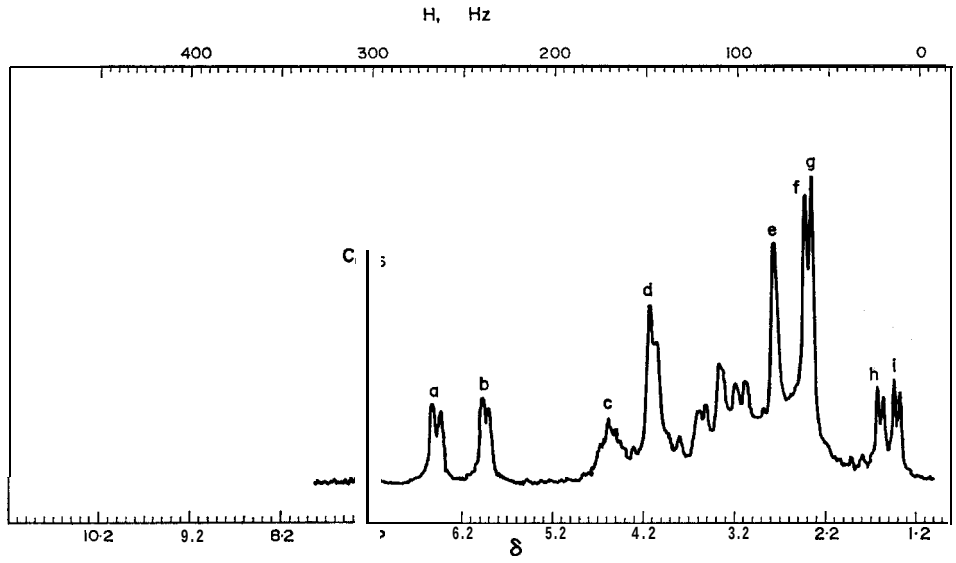


Peak	δ	Multiplicity*	J (Hz)	No. of Protons	Assignment
a	6.44	s		2	c-3
b	6.27	d	3	2	C-13'
c	6.19	C		2	c-13
d	5.62	d	3	2	C-13'
e	5.56	C		2	c-13
f	4.16	C		4	C-8, C-8'
g	3.57	S		4	C-2'
h	2.23	S		6	c-4, c-4 C-10' CH_3
i	2.17	S		6	
j	1.91	s		6	
k	1·38	d	7	3	C-10 CH,
l	1·24	d	7	3	C-10 CH_3

FIG. 1. 100 MHz NMR SPECTRUM ($CDCl_3$, TETRAMETHYLSILANE INTERNAL REFERENCE) OF FULVENE (4) FROM XANTHININ. THE PEAK AT δ 7·3 IS DUE TO THE SOLVENT, C_6H_6, USED FOR CRYSTALLIZATION. *S = SINGLET, d = DOUBLET, c = COMPLEX (OVERLAPPING DOUBLETS FOR PEAKS *c* AND e, OVERLAPPING MULTIPLETS FOR PEAK *f*.

for the methyl group at C-10, and because of the extensive prototropy involved in the generation of (5) from the lactonic precursor, it is to be expected that stereoisomerism at this position will be observed. Three broadened 6-proton singlets at S 1·91, 2·17 and 2·23 are assigned to the three pairs of vinyl methyl groups in the stereoisomers of (4). The singlet at S 3.57, integrating for four protons; is assigned to the methylene protons at C-2' of (4). A comparable model for this assignment has been described: in 3,6-dihydrochamazulene (12), the methylene protons at C-2 are found as a two-proton broadened singlet at S 3·0.[11] The C-S and C-8' protons (of CH—O of the lactone rings) appear as a 4-proton multiplet at about S 4.16. Since the original lactone in xanthinin is *trans*, it is probable that the same

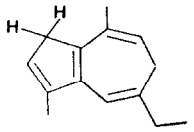
[11] D.J. BERTELLI and J. H. CRABTREE, *Tetrahedron* **24**, 2079 (1968).



Peak	δ	Multiplicity*	*J* (Hz)	No. of Protons	Assignment
a	6·55	c		4	C-13, C-13'
b	6·00	c		4	c-13, C-13'
c	4·67	c		4	C-8, C-8'
d	4.20	two singlets separated by 7 Hz		8	C-2', c-3
e	2.80	s		6	C-4, C-4', C-10' CH_3
f	2·47	s		6	
g	2.40	s		6	
h	1.63	d	7	3	C-10 CH_3
i	1.40	d	7	3	C-10 CH_3

FIG. 2. 100 MHz NMR SPECTRUM (CF_3CO_2H) OF CATION (5). THE INTERNAL REFERENCE IS C_6H_6 AT δ 7·28. *S = SINGLET, d = DOUBLET, C = COMPLEX (OVERLAPPING DOUBLETS FOR PEAKS a AND b, OVERLAPPING MULTIPLETS FOR PEAK C.)

configuration is present in (4). The characteristic a-methylene-y-la&one (C-13) protons are seen as a set of four 2-proton signals between δ 5·56 and 6·27. Of these, the two 2-proton signals at δ 6-27 and 5·62 can be assigned to the protons at C-13', and those at 6·19 and 5·56 to the protons at C-13. Since the C-13 protons are more influenced by the asymmetry at C-10 than are those at C-13', it is to be expected that the more complex signals at δ 6·19 and 5·56 are those of the former, and the relatively simpler signals at 6·27 and 5·62 are those of the protons well removed from C-10. The 2-proton singlet at δ 6·44 in Fig. 1 must be assigned to the C-3 protons. It will be seen that the NMR data are consistent with either of the two partial structures (13) and (14) (the former present in (4)). Structure (13) is chosen as the one in better agreement with the observed λ_{max} 388 nm, a value which is suggestive of a conjugated pentaene.



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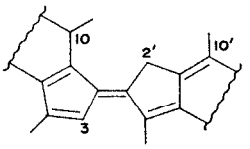

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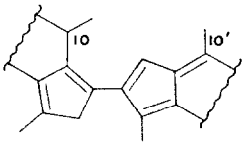


(14)

The NMR spectrum of the cation (5), prepared by dissolving the fulvene in trifluoroacetic acid, is shown in Fig. 2. The most conspicuous change from the spectrum of (4) is seen in the disappearance of the singlet at δ 6·44 assigned to the C-3 proton in (4). In addition, the signal for the C-2' (methylene) protons of (4), seen at δ 3·57 in Fig. I, now appears as part of a pair of broad overlapping singlets centered at δ 4·20 and integrating for a total of eight protons. This is in accord with structure (5), in which, by protonation of (4) are found two pairs of —CH_2— groups (at C-2' and C-3) in each of the stereoisomers. Confirmation of the assignment of the δ 4·20 signals to the C-2' and C-3 protons of the ion was found in a deuterium exchange experiment. The spectrum of (5), formed in deuterotrifluoroacetic acid and measured 40 min after preparation, showed complete loss of the S-proton set of signals at δ 4.20. No other protons were exchanged.

The methyl groups at C-4, C-4' and C-10' of (5) are seen as broadened 6-proton singlets at δ 2·40, 2·47 and 2·80. These chemical shifts are typical of those observed in polyenylic cations.[7,12] Other signals in the spectrum of (5) are similar to those seen in the spectrum of (4), except for some loss of resolution and a general downfield shift,* the latter due in part to the solvent used. It is to be expected that protons contiguous to the carbonium system would be deshielded to some degree. The C-8 and C-8' (lactonic) protons are seen as a 4-proton multiplet at δ 4.67. The 3-proton doublets (both J = 7 Hz) at δ 1·40 and 1·63 are assigned to the C-10 (epimeric) methyl groups, and the complex 4-proton signals at δ 6·00 and 6·55 are characteristic of the protons of the exocyclic methylene groups of the lactones.

Hydrogenation of the fulvene (4) at atmospheric pressure gave a colorless decahydro compound in which two double bonds, resistant to hydrogenation, have persisted, a conclusion in accord with the appearance of the molecular ion at *m/e* 466 and the composition $C_{30}H_{42}O_4$ found by high resolution mass spectrometry. The lack of significant absorption in the UV showed that the two double bonds were not in conjugation and that the exocyclic methylene groups of the lactone rings had been reduced. The IR spectrum contained a single strong peak at 1765 cm^{-1} (γ-lactone), and showed no absorption in the double-bond region.

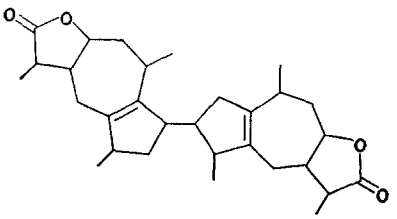
The NMR spectrum of the hydrogenation product showed no signals for protons in the olefinic region. Besides the expected signals for the lactonic (C-8) protons at about δ 4·0, no other signals were seen at chemical shifts lower than a high-field complex, those for the methyl groups appearing as a complex mass of overlapping doublets at about δ 0·9 and 1.27. That double bonds were still present in the hydrogenation product was shown by a positive test with tetranitromethane. The lack of methyl groups in the vinyl-methyl (CH_3—C=) region and the complete absence of vinyl protons require that the double bonds are found at the C-I/C-5 and C-l'/C-5' positions. It is known that the double bond found at the

[12] N. C. DENO, C. N. PITTMAN, JR. and J. D. TURNER, *J. Am. Chem. Soc. 87, 2153 (1963).*

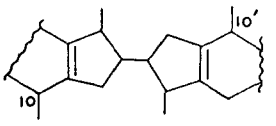
* Some of the loss of definition in the NMR spectrum of *(5)* was due to the formation of a trace of insoluble polymeric material in the NMR sample tube.

corresponding position in guaiol is resistant to hydrogenation.[13,14] These observations lead to the formulation of the hydrogenated fulvene as (15).

It is of special significance that the base peak in the mass spectrum of (15) is at *m/e* 233, indicating a halving of the molecule. This supports structure (15) as opposed to another structure (16).* It is apparent that the cleavage of (15) leads directly to a stabilized allylic cation of *m/e* 233. A corresponding fragmentation of (16), while not impossible, is not aided by corresponding stabilizing factors.



(15)



(16)

Among other sesquiterpene lactones that give the same color reaction shown by xanthinin is cumambrin-B (17).†[15,16] Treatment of cumambrin-B with hydrochloric acid gives a red solution with a λ_{max} identical with that formed from xanthinin. Neutralization of the solution leads to isolation of the fulvene (4), identical with that formed from xanthinin. It is to be noted that while the red cation formed from xanthinin retains the unaltered lactone groupings, opening of the lactone ring of cumambrin-B is an essential step in the formation of (5). These transformations are formulated in Scheme 1.‡

'Dimerization' by attack of the dienylic cation (20) upon its conjugate triene (19) leads to an intermediate tetraenylic cation (21) which, by prototropic changes, forms the final, stablized conjugated tetraenylic cation (4). It is apparent that the eventual formation of the —CH(CH$_3$)— grouping at a C-10 position will lead to the generation of diastereomeric ions which differ in configuration at that point.5

Numerous of the lactones of which (I), (3) and (17) are representatives give the same color reaction. It will be shown that the formation of the red cation (4)‖ is always

[13] P. A. PLOTTERN and L. LEMAY, *Helv. Chim. Acta* **23**, *897 (1940).*
[14] E. L. EISENBRAUN, T. GEORGE, B. RINIKER and C. DJERASSI, *J. Am. Chem. Soc. 82, 3648 (1968).*
[15] J. ROMO, A. ROMO DE VIVAR and F. DIAZ, *Tetrahedron 24, 5625 (1968).*
[16] M. A. IRWIN and T. A. GEISSMAN, *Phytochem. 8, 305 (1969).*

* Structures for the fulvene and the cation corresponding to the C-3/C-3' linkage of (16) have not been discussed in detail, although many of the foregoing spectral data can be accommodated to them as well as to (4) and (5). These alternative structures are regarded as less likely than (4) and (5) because of the ready formation of the ion *m/e 233.*

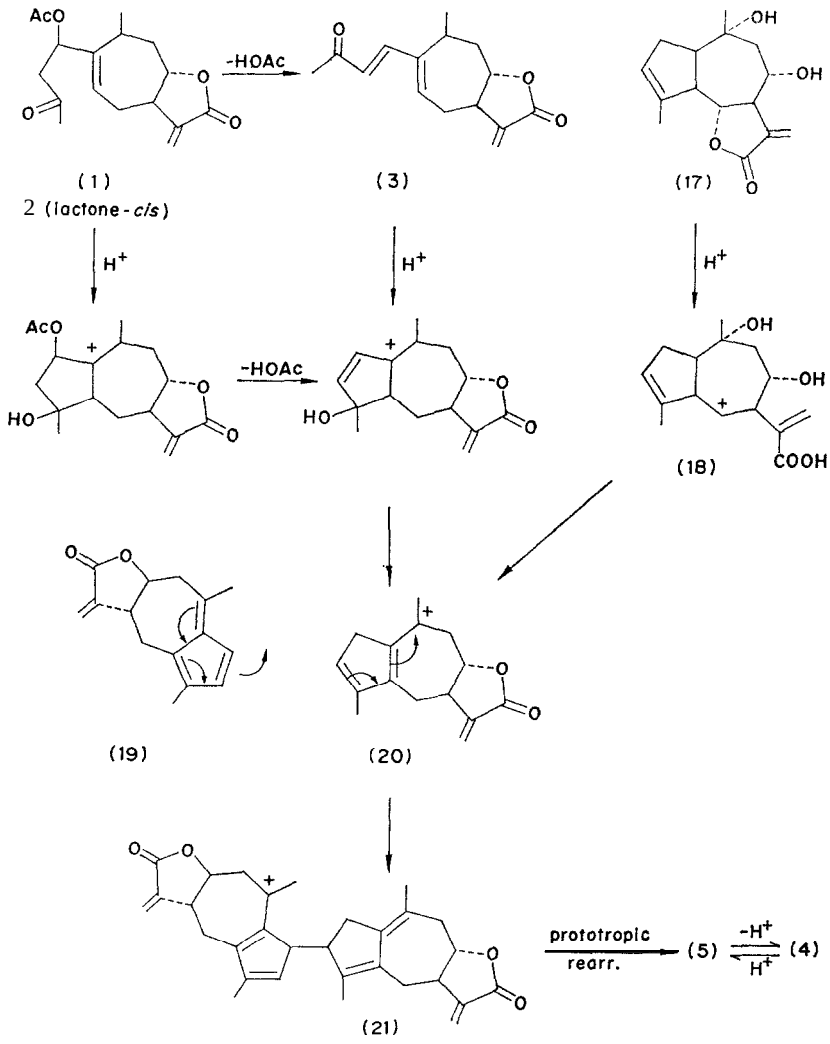
† Cumambrin-A, the acetate at C-8 of cumambrin-B, gives the same color reaction, as does 8-deoxycumambrin.

‡ The order in which the protonation, dehydration, etc., steps occur is arbitrary. The reaction is written in terms of a single, formal structure for the cation for simplicity of presentation.

§ The principal significance of the fact that xanthinin and cumambrin-B give identical compounds is that the final product is a guaianolide, formed from xanthinin by the ring-closure formulated in Scheme 1. The alternative-that a ring cleavage of cumambrin-B occurs at C-4/C-5-is quite unlikely. Further evidence on this point is to be described."

‖ Or minor variants, differing in details that do not affect the chromophore. For instance, if the lactone is opened as in the step (17) → (18) but does not reclose, the chromophore will be unaffected, although the product will not be (4).

SCHEME 1.



accounted for by the structural capability of forming an intermediate dienylic cation, or a triene, corresponding to (19) or (20) in Scheme 1, with participation of the lactone ring (as in (17)→(20)) when this is required and is closed in the C-6/C-7 position.

EXPERIMENTAL

UV spectra were measured on a Cary recording spectrophotometer Model 14, and IR spectra were measured on Perkin-Elmer Models 237 and 421 spectrophotometers. The NMR spectra were recorded on Varian Associates A-60D and HA-100 instruments. Descriptions of the relevant features of the NMR spectra are found in the Results and Discussion Section. Mass spectra were measured on an AEI-MS-9 mass spectrometer. Baker silica gel was used for column chromatography and Merck F_{254} precoated 5 x 10 cm silica gel plates were used for TLC.

Fulvene (4) from xanthinin (1) or ***xanthatin*** **(3),** The manipulations described here should be carried out as quickly as possible with minimum delay between stages. Excessive handling results in rapid deterioration of the product, which must be kept free of acid and stored at 0". Solutions of (4) in $CHCl_3$ can be stabilized and preserved with 0·5% triethylamine. Either xanthinin or xanthatin was a satisfactory starting material for preparationof (4); in practice 1 :1 (w/w) mixture of the two compounds were often used.

In a typical experiment, a mixture of xanthinin (500 mg) and xanthatin (500 mg) was refluxed for 5 mm in conc. HCl (200 ml) (there was considerable foaming). After cooling, the deep red solution was washed with $CHCl_3$ (2 x 100 ml). The acid solution was then added to a rapidly stirred mixture of 662 g K_2CO_3 in 1 1. H_2O, and 100 ml $CHCl_3$ kept below 5" during the addition. At the end of the addition (40 mm) the H_2O layer was yellow (pH > 8); the $CHCl_3$ layer was deep orange. After separation, the H_2O layer was extracted with 100 ml $CHCl_3$, and the combined $CHCl_3$ washed (H_2O) and dried (Na_2SO_4). Triethylamine (1 ml) was added to the $CHCl_3$ solution while drying. Solvent was removed under vacuum at room temp. and crystallization of the residual oil occurred after the addition of a little benzene. The powdery orange crystals were collected, washed with benzene, and dried under high vacuum at room temp. (3 hr) before storage at 0". The yield of product was 300 mg: m.p. 235-240" (capillary insertion into a preheated bath); IR ($CHCl_3$) 1760 (γ-lactone) 1567 cm-i (double bonds); UV max ($CHCl_3$) 388 (ϵ 30,000) and 248 nm (ϵ 8600); UV max (dioxane) 387 (ϵ 34,200) 247 (ϵ 5700), and 220 nm (end absorption); UV maxima of the protonated species in acidic solvents are reported in the Results and Discussion Section; mass spectra of samples from separate experiments (direct insertion probe, 70 eV); (1) probe temp. 215°, *m/e* (relative intensity), 456 (100) $[M]^+$, 441 (15) $[M\text{-}CH_3]^+$; (2) probe temp. 245°, *m/e* (rel. intensity), 456 (56) $[M]^+$, 441 (11) $[M\text{-}CH_3]^+$, 86 (100). (Calc. for $C_{30}H_{32}O_4$: C, 78.92; H, 7.06. Found: C, 79.15; H, 7.33; C, 78.85; H, 7.23; C, 78.76; H, 7.17 %.)

Fulvene **(4)** ***from cumambrin-B (17).*** Cumambrin-B (1 g) was treated according to the procedure described for the preparation from xanthinin, except that Et_2O rather than $CHCl_3$ was used to extract the product. The product crystallized from benzene as tiny dark orange-red prisms. The crystals were collected, washed with benzene, dried 1·5 hr at room temp. under high vacuum, and stored at 0". A TLC of the product was identical with that of (4) from xanthinin; its protonated form gave a visible λ_{max} 541 nm ϵ 98,000 (conc. HCl); the IR spectrum ($CHCl_3$) was superimposable on a spectrum of (4) prepared from xanthinin.

Hydrogenation product (15). The fulvene (4) (combined crystals and mother liquors) prepared from 1 g of xanthinin was hydrogenated in 100 ml ethyl acetate with 100 mg of 10%-Pd/C catalyst for 3 hr (1 atm., room temp.). After filtration through Celite and removal of solvent, the residue was chromatographed twice over silica gel (2 x 27 cm and 1 x 46 cm columns, both eluted with 1:1 v/v $CHCl_3$-benzene). The resulting colorless oil did not crystallize and was observed to darken (yellow, then orange) on standing at room temp.

The oil (120 mg) gave a positive (yellow) tetranitromethane test. It had IR bands ($CHCl_3$) at 1760 (γ-lactone), 1454, 1380 cm-'; UV max (EtOH), end absorption only; mass spectra of samples from separate experiments (direct insertion probe., 70 eV); (1) probe temp. 190°, *m/e* (rel. intensity), 466 (42) $[M]^+$, 451 (7) $[M\text{-}CH_3]^+$, 233 (100) $[C_{15}H_{21}O_2]^+$; (2) probe temp. 150°, *m/e* (rel. intensity), 466 (34) $[M]^+$, 451 (6) $[M\text{-}CH_3]^+$, 233 (100) $[C_{15}H_{21}O_2]^+$. ***Composition*** by highresolutionmassspectrum: Calc. mol. wt. for $C_{30}H_{42}O_4$: 466.308282; Found: 466.3083 ± 0.0002.

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