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SESQUITERPENE LACTONES: ACID-CATALYSED COLOR REACTIONS AS AN AID IN STRUCTURE DETERMINATION”?

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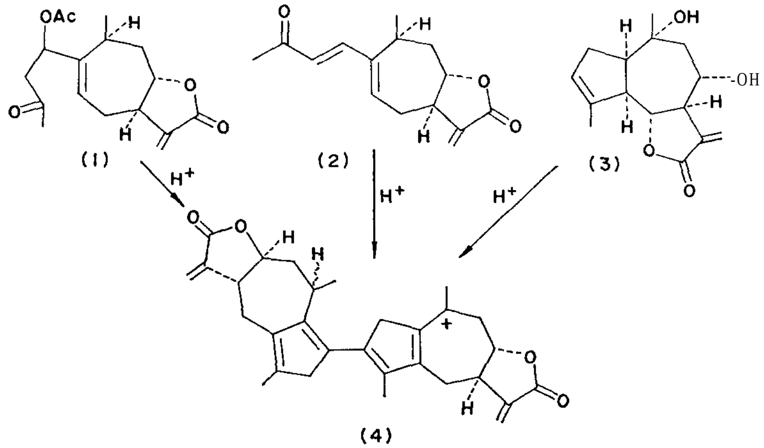
Abstract-Red and blue color reactions produced by certain sesquiterpene lactones in strong acid have been investigated. The results indicate that the color reactions can be correlated with a number of structural requirements.

INTRODUCTION

DURING the course of our studies of sesquiterpene lactones isolated from plants of the Compositae, we have observed that certain of these compounds yielded striking colors in strong acids.[1,2]

Except for indeterminate yellow to brown colors, with which this account is not concerned, the characteristic colors formed in these reactions are either a deep burgundy red with an absorption maximum at about 545 nm or a deep blue with an absorption maximum at about 595 nm. The colors are moderately stable in acids, persisting for several hours at room temperature and for much longer periods of time near 0”. Neutralization of



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* Contribution No. 2745 from the Department of Chemistry, U.C.L.A.

† Abstracted in part from the Ph.D. Dissertation of T. S. Griffin, U.C.L.A., 1970.

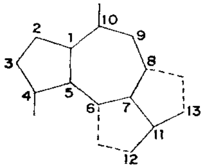
[1] A. YOSHITAKE and T. A. GEISSMAN, *Phytochem.* 8, 1753 (1969).

[2] T. G. WADDELL and T. A. GEISSMAN, *Phytochem.* **8, 2371** (1969).

the acid solutions results in formation of yellow to orange solutions from which the red or blue colors can be regenerated by acidification.

In an accompanying publication we have shown that the red chromophore generated from xanthinin (1), xanthatin (2), or cumambrin-B (3) in concentrated hydrochloric acid is a dimeric tetraenylic cation (4).[3]

The formation of (4) or similar dimeric cations was found to be dependent upon certain structural requirements in the parent sesquiterpene lactones. In order to delineate the scope and nature of these structural requirements, a study has been made of the acid color reactions of over 70 sesquiterpene lactones from five skeletal classes: guaianolides (5), xanthanolides (6), germacranolides (7), ambrosanolides (pseudoguaianolides) (8), and eudesmanolides (9). The structures of these are shown with dotted lines indicating alternative positions of lactone ring closure.



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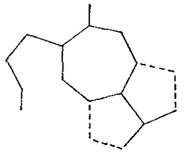

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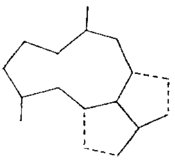

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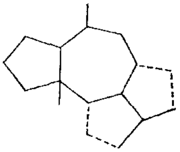

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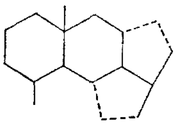

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RESULTS AND DISCUSSION

Illustrated in Chart 1 are three sesquiterpene lactones which gave red acid color reactions with absorption maxima near the λ_{max} 541 nm of cation (4). These lactones are the germacranolides baileyin (10)[2] and tatridin-A (11),[4] and the guaianolide pleniradin acetate (12).[1] The visible absorption maxima of the cations from (10), (11), and (12), generated and measured directly in concentrated hydrochloric acid, are compared in Table 1 with the maxima from xanthinin (I), xanthatin (2), and cumambrin-B (3). Included in Table 1 are the maxima of the cations from two cumambrin-B derivatives,[5,6] dihydrocumambrin-B (13, Chart 1) and cumambrin-B diacetate.

The near identity of the λ_{max} values in Table 1 suggest that the cations from the lactones are very closely related to or identical with cation (4). There is some variation in the ϵ values which can be ascribed to the different pathways that are required from the parent lactones to the same red cation. Each of the red cations from the compounds in Table 1 behaved similarly when neutralized. The neutral product was extractable from basic solution into ether, indicating that the lactone ring remained closed; and except for pleniradin acetate, each neutral product gave a spot on TLC identical with that of the fulvene from xanthinin[3]

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[3] T. S. GRIFFIN, T. A. GEISSMAN and T. E. WINTERS, *Phytochem.* 10, 2487 *(1971).*

[4] M. A. IRWIN and T. A. GEISSMAN, unpublished results.

[5] M. A. IRWIN and T. A. GEISSMAN, *Phyiochem. 8, 305 (1969).*

[6] J. ROMO, A. ROMO DE VIVAR and E. DIAZ, *Tetrahedron 24, 5625 (1968).*

TABLE 1. VISIBLE ABSORPTION MAXIMA IN ACID*

Compound	λ_{max} (nm)	ϵ x 10^{-4}
Xanthinin (1)	541	3·0
Xanthatin (2)	540	2.3
Cumambrin-B (3)	541	0·96
Baileyin (10)	542	1·6
Tatridin-A (11)	542	2·0
Pleniradin acetate (12)	542	1.5
Dihydrocumambrin-B (13)	541	3.2
Cumambrin-B diacetate	541	0.88

* All spectra measured in conc. HCl with heating, except baileyin (10), which was measured after 1 hr at room temp. by Dr. T. G. Waddell.

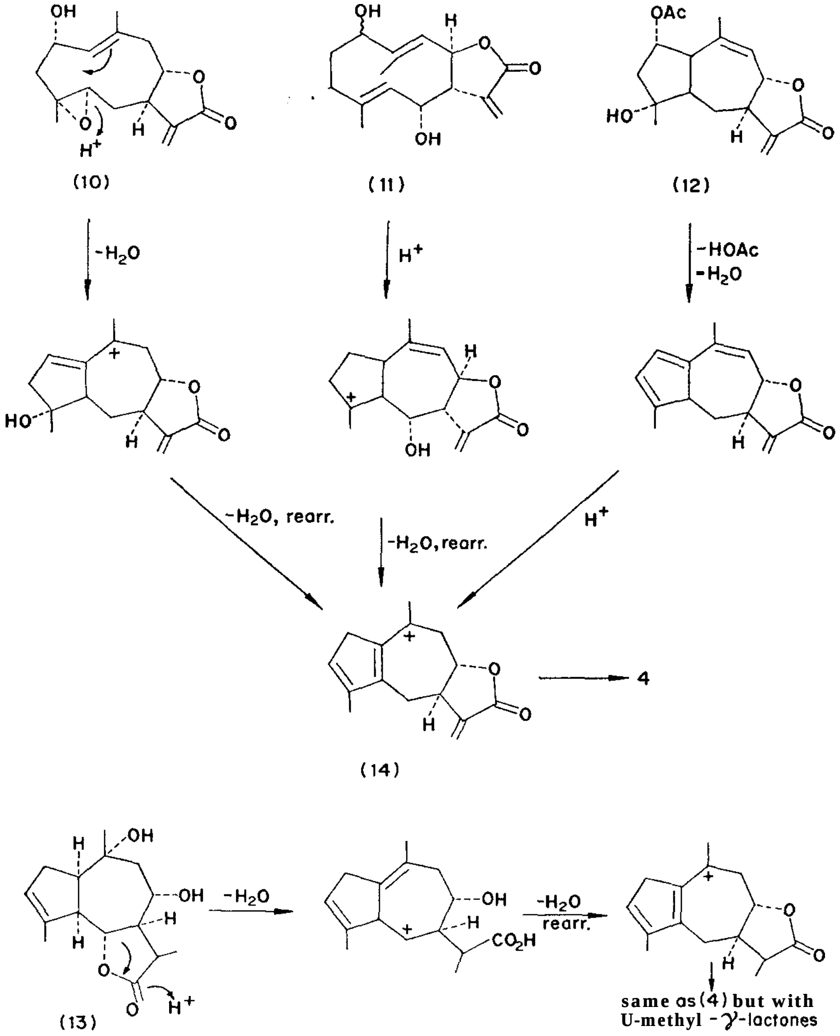
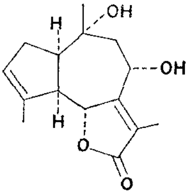


CHART 1.

(R_f ~0·6; $CHCl_3$–5% acetone, bright pink spot on conc. H_2SO_4 spray). The TLC spot of the pleniradin acetate product (also bright pink) was at R_f ~0·5 and may have been affected by considerable decomposition material which was in evidence at low R_f. Isolation of the neutral fulvenes from baileyin, tatridin-A, pleniradin acetate, and the cumambrin-B derivatives for direct comparison with the xanthinin fulvene was not possible due to the limited availability of these compounds. However, the similarity of the TLC spots suggests that the fulvenes are closely related or identical.

Possible pathways from baileyin (IO), tatridin-A (11), and pleniradin acetate (12), to a dienylic cation (14), the postulated precursor to cation (4),[3] are shown in Chart 1.* Tatridin-A, unconventionally represented to show a possible mode of cyclization, has been tentatively assigned structure (1 1).[4] In order to formulate the cyclization, the 4,5-double bond must isomerize; apparently the dienylic cation and resulting red cation are enantiomeric with (14) and (4). Also contained in Chart 1 is a possible pathway from dihydrocumambrin-B (13) to a cation which should have the same structure as (4), except that it possesses a-methyl-y-lactones, a condition which does not affect the λ_{max} of the cation.

It is of interest to note the large difference in ϵ values measured for cumambrin-B (9 x 10^3) and for dihydrocumambrin-B ($3{\cdot}2 \times 10^4$). Moreover, isocumambrin-B(15),[4] which has the 7,11-endocyclic double bond, did not give a red color in acid but a yellow (λ_{max} 384 nm) instead. This demonstrates that acid-catalysed opening of the y-lactone by elimination (shown in Chart 1 with 13) is hindered or prevented by the exo- or endoconjugated double bond, possibly due to the formation of alternatively stabilized chromophores.



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The sesquiterpene lactones (Chart 2) dihydroparthenolide (16),†,[7] 3-deoxoisophotosantonic lactone (17), and 8-deoxycumambrin-B (18) yielded red colors in acid, but with absorption maxima near 548 nm. When the red cations from these compounds were neutralized, the neutral product could not be extracted from basic solution, indicating that the γ-lactone had opened to an acid. The visible absorption maxima of the cations are summarized in Table 2. From the position of the absorption maxima (548 nm), it is expected that the red chromophore is a cation with four conjugated double bonds,[3,8] e.g., a dimeric tetraenylic cation, similar in structure to (4), except that the compounds are carboxylic acids instead of y-lactones.

Chart 2 summarizes possible pathways[6] to a red cation (λ_{max} 548 nm) to which structure (20) is assigned. The only structural difference between (20) as shown and (4) is the presence of two carboxylic acid groups rather than two y-lactones. The 5 nm shift in λ_{max} may be

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* The order in which the steps occur is arbitrary. Only one of the possible resonance contributors to the structure of the hypothetical cationic intermediates are shown; *rearr.* refers to prototropic double bond rearrangements.

† Parthenolide (11,13-exocyclic double bond) gave the same color reaction, λ_{max} 548 nm.

[7] T. R. GOVINDACHARI, B. S. JOSHI and V. N. KAMAT, Tetrahedron 21, 1509 (1965).

[8] P. BLATZ, D. PIPPERT, L. SHERMAN and V. BALASUBRAMANIYAN, *J. Chem. Educ.* 46, 512 (1969).

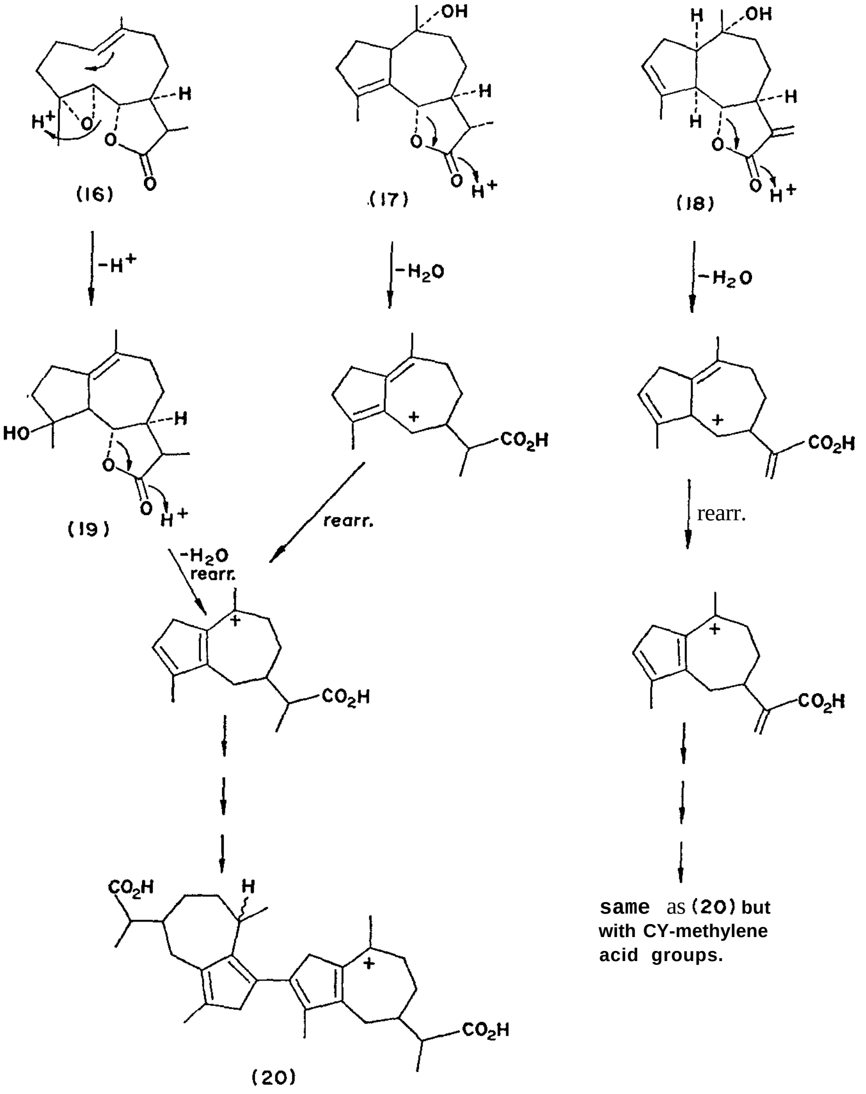


CHART 2.

TABLE 2. VISIBLE ABSORPTION MAXIMA IN CONC. HCl*

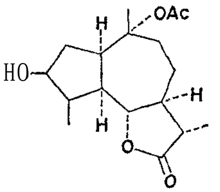
Compound	λ_{max} (nm)	ϵ x 10^{-4}
Dihydroparthenolide (16)	547	2·1
3-Deoxoisophotosantonic (17) Lactone	547	4·3
8-Deoxycumambrin-B (18)	549	2.2

* All spectra measured after heating.

related to this difference, although the specific cause is not clear. The guaianolide (19), shown as an intermediate between (16) and (20), was reported to have been isolated from (16) following cyclization under mildly acidic conditions.[7] The cation from 8-deoxycumambrin-B (18) would be expected to have the same structure as (20), except that it would contain the a-methylene grouping.

As shown in Charts 1 and 2, in each of the guaianolides which yielded red cations there were present three functional groups (hydroxyl, acetoxyl, double bond, or C-6/C-7 γ-lactone) which could give rise to a guaianolide containing three double bonds or an equivalent dienylic cation. In the germacranolides (and xanthanolides)[3] three functional groups equivalent to double bonds were present following cyclization. These 'double bond equivalents' in the above examples did not involve the C-8 y-lactones, which remained closed, nor C-8 hydroxyl or acetoxyl groups which participated in reclosure of C-6 y-lactones at C-8.

Three guaianolides which gave red acid color reactions of moderately weak intensity were tetrahydroisophotosantonic lactone acetate (21, λ_{max} 548 nm, ϵ 3200), tetrahydrocumambrin-B[1-6] (22a, λ_{max} 5 4 3 nm, ϵ 3000), and tetrahydrocumambrin-B acetate[4-6]



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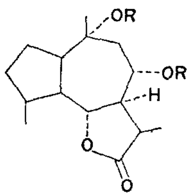

(22a) R = H
(22b) R = Ac

(22b, λ_{max} 542 nm, ϵ 1000). From the maximum at 548 nm, the cation from (21) is expected to have the same structure as cation (20) (Chart 2), but the low value of ϵ indicates that cation formation is not as complete as in guaianolides with a double bond (3,4 or 4,5) in the 5-membered ring. In structures (22a) and (22b) the third 'double bond equivalent' is necessarily located at C-8, so the low values may result from the fact that a double bond generated at C-8 must undergo extensive migration into the smaller ring to form a suitable intermediate for dimerization. The maxima ($\sim$ 542 nm) are more in accord with cation (4) than cation (20) although there is experimental evidence that the y-lactones had opened to acids.

Certain of the lactones of the class yield deep blue acid color reactions. Two of these are the guaianolides artabsin*,[9] (23; λ_{max}. 595 nm, ϵ not known),[10] and dihydroisophotosantonic lactone acetate (24; λ_{max} 596 nm, ϵ 17,000),[11] both shown in Chart 3. These structures contain four 'double bond equivalents'. Therefore, if reactions in acid developed according to the patterns suggested for the red cations, the resulting cation might have been expected to contain six conjugated double bonds. However, studies of linear polyenylic cations have shown that a cation with six linearily conjugated double bonds is blue-green with a λ_{max}

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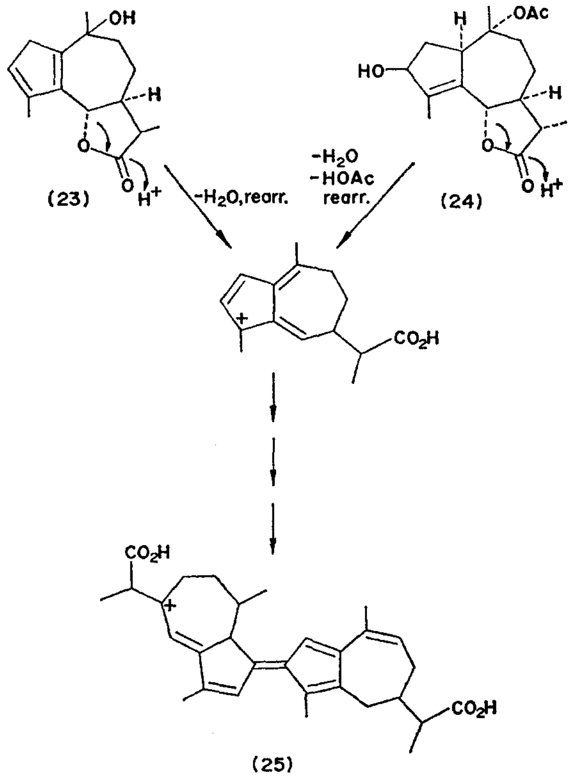
* Spectra were measured in 1 :1 (v/v) conc. HCl–ethanol after 1 hr at room temp. Insufficient artabsin was available for an accurate measurement; however, a very small quantity gave an instant deep blue, even if diluted with large volumes of acid, so it is probable that the value of ϵ is large.

[9] T. A. GEISSMAN and T. E. WINTERS, *Tetrahedron Letters 3145 (1968).*

[10] T. S. SORENSEN, *J. Am. Chem. Soc. 87, 5075* (1965).

[11] P. E. BLATZ and D. L. PIPPERT, *J. Am, Chem. Soc. 90, 1296 (1968).*

CHART 3.



between 662–702 nm, whereas a cation with five linearily conjugated double bonds is blue with a λ_{max} between 590–625 nm.[8,11] The blue cations from (23) and (24) clearly fall within the 590–625 nm range, and so the sixth double bond must be cross-conjugated with a cation containing five linearily conjugated double bonds. It has been shown that a cross-conjugated double bond has little effect upon the λ_{max} of a linearily conjugated carbonium ion (e.g. the trivinylcarbonium ion).[12]

The above observations are summarized in Chart 3, in which the structure for the blue cation is represented as (25) by analogy with the red cations. A neutralization experiment demonstrated that the γ-lactones had opened to acids, showing that opening of the lactone played a part in the color formation.

It was observed that those guaianolides which contained keto or epoxy functionality did not give red or blue color reactions. Twelve keto-guaianolides were tested in acid with no positive results. The carbonyl groups were located at C-2, C-3, or at C-8 and would be expected to prevent formation of cationic dimers by blocking key carbons (C-2 or C-3) or by forming alternatively stabilized chromophores. Four epoxy-guaianolides were found to give only yellow–green colors in acid. The epoxides were either within the five-membered ring or one bond length away. A possible explanation for the lack of color from epoxides is that they undergo pinacol rearrangements in acid to form keto-guaianolides.

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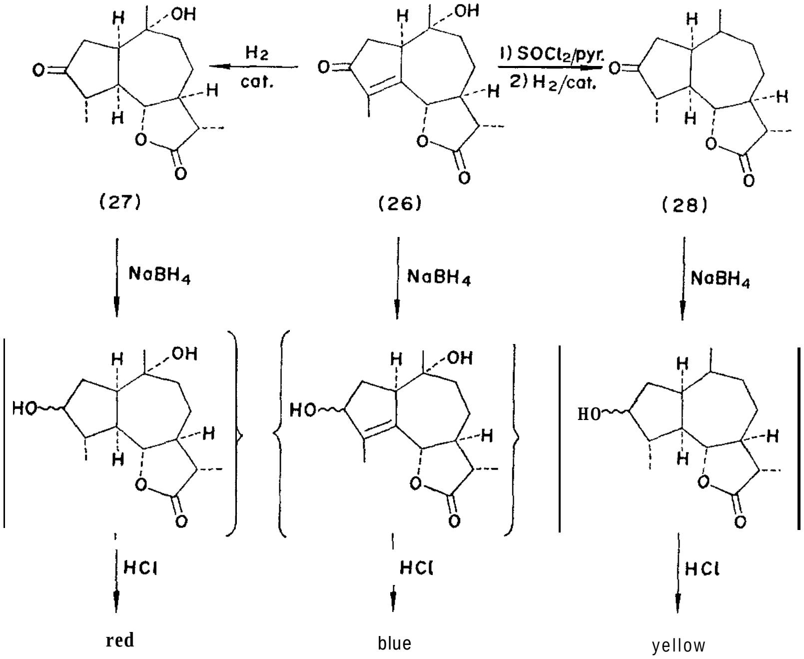
[12] T. S. SORENSEN, *Can. J. Chem.* 43, 2744 (1965).

As expected, certain keto-guaianolides, when reduced with sodium borohydride, give products which when treated with acid give red or blue colors. A series of experiments which provided clear confirmatory evidence for the observations described here was performed with the known guaianolide, isophotosantonic lactone (26)[13,14] and its reduction products, 4,5-dihydroisophotosantonic lactone (27) and tetrahydroanhydroisophotosantonic lactone (28). Reduction of these with sodium borohydride and acidification with HCI gave, respectively, blue, red and yellow colors. As shown in Chart 4, the reduction products of (26), (27) and (28) possess, respectively, four, three and two 'double-bond equivalents'.

Chart 4.



Twelve ambrosanolides (8) and fourteen eudesmanolides (9) were tested for the acid color reactions and in all cases the results were that no red or blue colors were produced. Several keto-ambrosanolides, e.g., parthenin,[15] and keto-eudesmanolides, e.g, artecalin acetate,[16] were treated with acid after reduction with sodium borohydride. No stable red or blue colors were observed in these reactions. It is evident that formation of guaianolide-type cationic dimers of the type (20), (25) or (4) would be precluded in ambrosanolides and eudesmanolides unless skeletal rearrangements occurred.

The examples in Charts 1 and 2 indicate that germacranolides give acid color reactions provided that cyclization to a guaianolide occurs, and that three or four double bond equivalents are present following cyclization. Germacranolides which cyclize to eudesmanolides do not give the color reactions. Examples are chamissonin diacetate[17] and

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[13] D. H. R. Barton, P. de Mayo and M. Shafiq, *J. Chem. Soc. 929 (1957).*

[14] D. H. R. Barton. J. E. D. Levisalles and J. T. Pinhey, *J. Chem. Soc.* 3472 (1962).

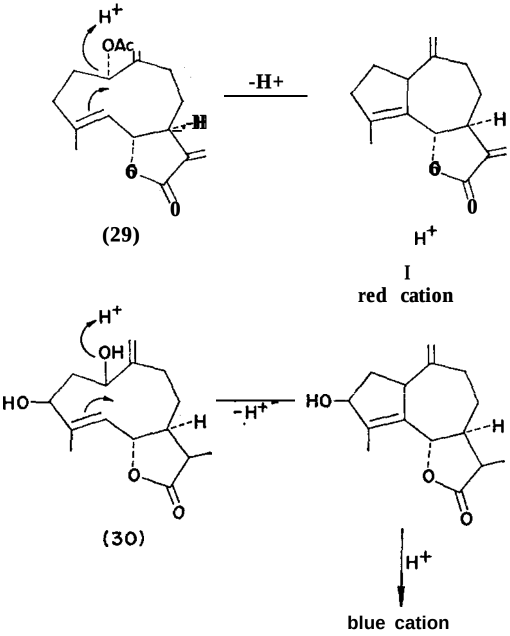
[15] W. Herz, H. Watanabe, M. Miyazaki and Y. Kishida, *J. Am. Chem. Soc.* 84, 2801 (1962).

[16] T. A. Geissman, T. S. Griffin and M. A. Irwin, *Phytochem. 8, 1297* (1969).

[17] M. F. L'Homme, T. A. Geissman, H. Yoshioka, T. H. Porter, W. Renolda nd T. J. Mabry, *Tetrahedron Letters 3161, (1969).*

dihydromethoxyeupatolide[18] which do not produce colors in acid, and are known to cyclize to **eudesmanolides.**[17,18] **Erioflorin**[19] gave a light yellow color in acid.

The germacranolides artemorin acetate (29)[20] and dihydroridentin (30)*[,21] produced red and blue colors, respectively, in acid. The λ_{max} values were those expected for red and blue cations, but the ϵ values were very low: artemorin acetate-h,,, 548 nm, ϵ 220; dihydroidentin-A,,, 594 nm, ϵ 75. This would indicate that these germacranolides contain the structural requirements for cyclization to guaianolides with three and four double bond equivalents respectively, but that alternative reactions may be occurring with greater ease.



(29)

I
red cation

(30)

blue cation

CONCLUSIONS

From the preceding discussion, it is evident that the color reactions can be an aid in structure determinations of sesquiterpene lactones. The tests have the advantage that they are rapidly and easily run, and require only minute quantities of compound. Of course, the empirical nature of the color reactions should be kept in mind when interpreting the color tests of new lactones. The following general remarks summarize the results of the color reaction tests.

(1) Guaianolides, and xanthanolides and germacranolides which cyclize to guaianolides, gave the color reactions provided that the correct number and type of functional groups (double bond equivalents) were present to generate the cationic dimers. Three double bond equivalents were necessary to generate a red cation (λ_{max} about 545 nm) and four double bond equivalents for a blue cation (A,,, about 595 nm).

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* **Ridentin (11,13-exocyclic double bond) gave a visually similar but somewhat less stable blue color.**

[18] **L. DOLEJS and V. HEROUT,** ***Coll. Czech. Chem. Commun.*** **27, 2655 (1962).**

[19] **S. J. TORRANCE, T. A. GEISSMAN and M. R. CHEDEKEL,** ***Phytochem.*** **8, 2381 (1969).**

[20] **T. A. GEISSMAN,** ***Phytochem.*** **in press (1970).**

[21] **M. A. IRWIN, K. H. LEE, R. F. SIMPSON and T. A. GEISSMAN,** ***Phytochem. 8, 2009*** **(1969).**

(2) No keto- or epoxy-guaianolide has been observed to give the color reactions. Reduction of certain keto-guaianolides with sodium borohydride followed by acidification produced the characteristic colors provided that the three or four double bond equivalents were present in the intermediate alcohols.

(3) No ambrosanolides or eudesmanolides were found to give the acid color reactions.

(4) Germacranolides which cyclized to eudesmanolides in acid did not form the colors. Certain germacranolides gave faint color reactions. These compounds contained the structural elements that allowed formation of limited amounts of the colored cations, but apparently acid-catalysed changes of other kinds occurred with greater ease.

EXPERIMENTAL

UV-visible spectra were measured on Cary recording spectrophotometers Models 11 and 14. The solvents were reagent grade conc. HCI or a 1 :1 (v/v) mixture of conc. HCl and EtOH. Merck F_{254} precoated 5 x 10 cm silica gel plates were used for TLC, developed with $CHCl_3$ or $CHCl_3$-5% acetone, and visualized by spraying with conc. H_2SO_4.

Color reaction procedures. Between 0·5 and 1·0 mg of a typical sesquiterpene lactone was dissolved in EtOH and an equal vol. of conc. HCI (10·00 ml gave concentration ranges (10^{-4}–10^{-5} M) suitable for absorption spectra). If the colored cation developed readily without heating, the spectrum was measured after 1 hr at room temp. In other cases the solution was heated to boiling for from 2 to 4 min and the spectrum measured immediately after cooling. The choice of acidic solvent for the maximum ϵ value (conc. HCI or 1 :1 conc. HCl-ethanol) was made by trial-and-error. Experiments showed that there was a maximum 6% solvent loss during heating and concomitant handling. Because of this and other unknown experimental factors, ϵ values are to be regarded as approximate (e.g. ±5–10%). The ϵ values were based upon concentrations of the parent lactones and do not take into account dimerization or other factors. For example, the ϵ value of cation (4) from xanthinin (1) is known to be $1·0 \times 10^5$.* The ϵ measured directly using the above procedure was 3×10^4, indicating that (4) was formed in approximately 60 % yield.

Isophotosantonic lactone (26) and its dihydro derivative (27) were prepared as described by Barton *et al.*[14] Anhydrotetrahydroisophotosantonic lactone (28) was prepared from (26) by dehydration ($SOCl_2$/pyridine) followed by hydrogenation. It had m.p. 197–200° (reported,“’ m.p. 195-200”). (Found: C, 72.06; H, 8.87. Calc. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86%).

3-*Deoxoisophotosantonic lactone* (17). This compound was prepared by hydrogenolysis of isophotosantonic lactone (17) while attempting to simply hydrogenate the 4,5-double bond. 1 g of (17) in 20 ml ethyl acetate with 150 mg 10%-Pd/C catalyst was hydrogenated overnight (1 atm.). Chromatography of the product over silica gel eluted with $CHCl_3$ yielded 55 mg of colorless prisms (from EtOAc), m.p. 145–148°, $[\alpha]_D^{27}$ +124°) (c = 0.54, CHCl-). IR peaks ($CHCl_3$) 3570, 1770, 1660 cm-‘. Mass spectrum (70 eV), *m/e* (rel. intensity) 250 (31) $[M]^+$, 232 (11) $[M-H_2O]^+$, 43 (100). (Found: C, 72.09, H, 8.67. Calc. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86%).

Dihydroisophotosantonic lactone acetate (24). Isophotosantonic lactone acetate (860 mg, prepared according to the procedures of Büchi *et al.*[22]) in 100 ml EtOH was reduced by dropwise addition (5 min, room temp.) of 270 mg $NaBH_4$ in 200 ml EtOH. The yellow solution was acidified (dil. HCl), the EtOH removed *in vacuo*, and the product taken up into $CHCl_3$. The $CHCl_3$ solution was washed (H_2O), dried ($MgSO_4$), evaporated, and the residue chromatographed over silica gel eluted with CH_3Cl–acetone, yielding 200 mg of fine white needles (from ether) m.p. 159–161°, $[\alpha]_D^{27}$ +37° (c = 1, $CHCl_3$). IR peaks ($CHCl_3$) at 3580, 1770, 1725 cm-‘. (Found: C, 66.27; H, 7.99. Calc. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85 %).

Tetrahydroisophotosantonic lactone acetate (21). Isophotosantonic lactone acetate[22] was hydrogenated (1 atm., 10%-Pd/C, EtOH) and the resulting mixture refluxed in EtOH with anhydrous NaOAc to prepare 4,5-dihydroisophotosantonic lactone acetate,[14] m.p. 167–168·5° (reported,[14] m.p. 165-167’). This product (1.85 g) was reduced with 1 g $NaBH_4$ in 100 ml EtOH (40 min, room temp.). The mixture was acidified (dil. HCl), the EtOH removed *in vacuo*, and the residue taken up into CHCI,. The $CHCl_3$ was washed (H_2O), dried ($MgSO_4$), evaporated, and the residue chromatographed over silica gel eluted with $CHCl_3$ yielding 58 mg of tiny white needles (from ether). The product (21) showed erratic melting points (1 10°–126”); data from NMR indicated ether of crystallization. Therefore, the sample was dried at 56” in a high vacuum for over 24 hr, m.p. 125-127”. IR peaks ($CHCl_3$) at 3570, 1760, 1725 cm-‘. (Found: C, 65.50; H, 8.46. Calc. for $C_{17}H_{26}O_5$: C, 65.78; H, 8.44%).

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* This value of ϵ for cation (4) is based upon the formation of (4) from the pure fulvene. See Ref. 4.

[22] G. BÜCHI, J. M. KAUFFMAN, and H. J. E. LOWENTHAL, *J.* Am. *Chem. Soc.* *88, 3403 (1966).*

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