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CEROALBOLINIC ACID A NEW ANTHRAQUINONE PIGMENT ISOLATED FROM *CEROPLASTES ALBOLINEATUS*

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Abstract—Ceroalbolinic acid, isolated from the insect *Ceroplastes albolineatus*, is an anthraquinone pigment related to carminic acid. Structure Ia has been assigned to it on the evidence further described.

Ceroplastes albolineatus[1] is a Coccidae, the females of which infest during the rainy season the shrub popularly known as "Palo loco" [*Senecio praecox*, (Cav)]. This shrub is widely distributed in the southern part of the Valley of Mexico. The females are covered by thick clusters of wax. Treatment with chloroform dissolves the wax which is being studied at present.[2] From the ethanolic extracts of the wax-free insects a red substance was isolated which was named ceroalbolinic acid. This acid (Ia, $C_{16}H_{10}O_8$) is optically inactive. It begins to decompose at 290° and carbonizes without melting. This substance gives a positive ferric chloride test and dissolves in alkali with a green colour, which slowly darkens. Its IR spectrum shows bands at 3500 (hydroxyl groups), 1725 (carboxyl group), 1643 (unsaturated carbonyl groups) and 1590 cm^{-1} (C—C double bonds).

Acetylation of ceroalbolinic acid with acetic anhydride and pyridine did not yield an homogeneous product, while perchloric acid catalyzed acetylation afforded a pale yellow tetraacetate (Ib, $C_{24}H_{18}O_{12}$) which shows in the IR a strong band at 1790 cm^{-1} (acetyl groups); a band at 2900 cm^{-1} (broad) and another one at 1735 cm^{-1} which can be attributed to a carbonyl group.

The tetraacetate (Ib) is soluble in aqueous sodium bicarbonate solution and reacts with ethereal diazomethane yielding a monomethyl ester (Ic) ($C_{25}H_{20}O_{12}$). Its IR spectrum shows an ester carbonyl band at 1735 cm^{-1}.

A treatment of ceroalbolinic acid with ethereal diazomethane solution afforded a trimethyl ether of ceroalbolinic methyl ester (Id). The remaining phenolic hydroxyl must be associated with a carbonyl group, since it is known that these phenols are not methylated by this procedure. This assumption is confirmed by the IR spectrum of Id, which shows a band at 1630 cm^{-1} attributed to a chelated α,β-unsaturated carbonyl group. The trimethyl ether (Id) is partially soluble in aqueous sodium hydroxide and yields a monoacetate (Ie) by acetylation in the presence of perchloric acid. The monoacetate (Ie) has an IR band at 1768 cm^{-1} (phenolic acetate group) and does not show the band at 1630 cm^{-1}. Saponification of the methyl ester (Id) furnished the acid Ig.

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[1] I am grateful to Dra. Leonila Vázquez of the Instituto de Biología for the classification of the insects.
[2] Tirso Ríos and F. Colunga, *Chem. & Ind.* 1184 (1965).

Methylation of ceroalbolinic acid, Ia, with methyl sulphate in acetone, yielded the tetramethylether of ceroalbolinic methyl ester (If). Saponification of this product afforded the acid Ih. Reductive acetylation of the acetate (Ie), yielded the hydroquinone diacetate (II) whose solutions possess an intense green fluorescence. Its IR spectrum does not show the bands due to the quinonoid chromophore (1643 cm^{-1}).

It is known that reductive acetylation of quinones gives products which show the characteristic UV absorption of the related hydrocarbons.[3] The leucoacetate (II), obtained by reductive acetylation of Id, shows λ_{max} 277, 349, 368 and 407 mμ which are present in the UV described for a substituted anthracene.[4] Furthermore when the trimethyl ether methyl ester of ceroalbolinic acid (Id) was distilled over Zn 1-methylanthracene was obtained.

The chemical and spectroscopic evidence cited above, indicates that the eight oxygen atoms of ceroalbolinic acid are distributed in a methyl tetrahydroxy-carboxy anthraquinone with one of the hydroxyl groups chelated with a carbonyl group of the quinonoid chromophore.

It is interesting to point out that similar compounds such as carminic, kermessic and laccaic acids, have been previously isolated from coccidae.[5]

Confirmation of the above statements and information concerning the relative position of the substituents were obtained in the following manner.

Decarboxylation of ceroalbolinic acid (Ia) in quinoline solution with copper chromite, yielded a tetrahydroxy methylanthraquinone, characterized as its tetraacetate (IIIa) by acetylation in the presence of perchloric acid. Methylation with diazomethane afforded the trimethyl ether (IIIb).

The same derivative was obtained by saponification of the trimethylether of ceroalbolinic acid methyl ester (Id) which yielded the acid (Ig), and decarboxylation of the latter with quinoline and copper chromite.

Potassium permanganate oxidation of the trimethyl ether of ceroalbolinic acid methyl ester (Id), yielded a tricarboxylic acid monomethyl ester monomethyl ether, ($C_{12}H_{12}O_7$). C-Methyl and methoxyl determinations indicate the presence of one C-methyl and one methoxyl group (confirmed by NMR). The anhydride and the trimethyl ester (IVb) was prepared by the standard methods. A comparison of this compound with an authentic sample* of cochenillic acid methyl ether trimethylester, obtained from carminic acid, proved to be identical by mixed m.p., IR and NMR spectra.

The formation of cochenillic acid[6] (IVa) places the methyl at C-1, a hydroxy at C-3 and the carboxyl group at C-2. The location of the three hydroxyl groups in the other anthraquinone ring can be distributed in positions 5, 6, 7 or 6, 7, 8; the sequences 5, 6, 8 or 5, 7, 8 are excluded, since the IR spectra of ceroalbolinic acid and its derivatives Id, Ig and IIIb indicate the presence of only one chelated carbonyl group; furthermore methylation of Ia with dizomethane left only one free hydroxyl group, and it is

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* We thank Dr. Karl Dimroth from the University of Marburg for generous gift of samples of cochenillic acid.

[3] B. R. Brown, A. W. Johnson, J. R. Quayl and A. R. Todd, *J. Chem. Soc.* 110 (1954).

[4] B. H. Howard and H. Raistrick, *Biochem. J.* 482 (1955); R. Norman Jones, *Chem. Rev.* 353 (1941).

[5] R. H. Thomson, *Naturally Occurring Quinones* p. 222 (1959).

[6] J. C. Overeem and G. J. M. Van Der Kerk, *Rec. Trav. Chim.* **83**, 1023 (1965).

known that the hydroxyl groups chelated with the anthraquinone carbonyl groups are not methylated by this procedure.

The ceroalbolinic acid is remarkably unstable in strong alkali, its purple solution in aqueous sodium hydroxide when exposed to air becomes orange in a few seconds and then colourless. Anthragallol and rufigallic acid, in which there are three hydroxyl groups in 1, 2, 3 position, behave in this way.

A comparative study of the NMR spectra of some derivatives of ceroalbolinic acid (Table 1) permits the assignment of the positions 5, 6 and 7 for the free hydroxyl groups.

TABLE 1.* EXPRESSED IN PPM

	—CH$_3$(1)	MeO(3)	—COOCH$_3$(2)	H(4)	H(2)	H(8)	—OH
Id	2·69	3·95	4·01	7·33		7·66	12.9
IIIb	2·75	3·92, 4·0		7·31	6·95	7·57	13·05
IVb	2·18	3·80	3·85	7·28			

* The spectra were determined on a Varian A-60 spectrometer in CDCl$_3$ solution using tetramethylsilane as internal standard. The integration curves are in accord with the proton distribution indicated by their formulae.

Since the chemical environment of the aromatic proton (signal at 7·28 ppm) of the derivative of cochenillic acid IVb is very similar to that of the C-4 protons of the trimethyl ethers (Id and IIIb), the signals exhibited by these derivatives at 7·33 and 7·31 ppm, respectively, were ascribed to the C-4 protons. Therefore the signals at 7·66 and at 7·57 ppm of the trimethyl ethers (Id and IIIb) correspond to the C-8 protons, and the signal at 6·95 ppm in IIIb can be ascribed to the C-2 proton.

If the trimethyl ether (Id) is represented by structure V, the C-4 and C-5 protons due to a similar chemical environment should exhibit in the NMR spectrum only one signal for the two protons. Therefore, structure Ia is proposed for ceroalbolinic acid.

EXPERIMENTAL*

Isolation of ceroalbolinic acid (Ia). The lumps of wax containing the insects, were collected in the Pedregal de San Angel in the vicinity of Mexico City during June and July (1960). The insects and their wax (120 Kg) were gently expressed to eliminate water. The dark red aqueous fraction contained some pigment due to the destruction of some insects. It was acidified with conc. HCl and thoroughly extracted with ether. Evaporation to dryness left a solid residue. The expressed wax was treated with CHCl$_3$ at room temp with stirring until dissolution, leaving the insects as a residue. The chloroform solution was filtered off and evaporated to dryness.[7]

The insects were crushed and extracted with boiling EtOH until the extract was colourless. The EtOH solution was concentrated to a small volume and made alkaline with a 10% NaOHaq; it was then diluted with water and extracted with ether. The ethereal extract was discarded. The aqueous fraction was acidified with conc HCl acid and extracted thoroughly with ether. The organic extract was evaporated to dryness. The residue was combined with that obtained from the ether extraction of the original aqueous fraction. The solid material was washed with hexane and benzene, and then

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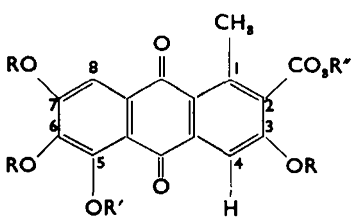
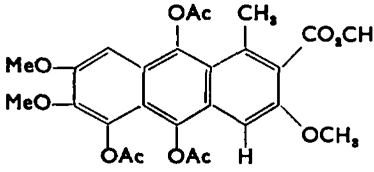
* M.ps were determined on a Kofler block and are uncorrected. The UV absorption spectra were determined in 95% EtOH solution with a Beckman DK2 spectrophotometer. The IR spectra were run in CHCl$_3$ solution unless otherwise noted, on a Perkin–Elmer double beam spectrophotometer. Microanalyses and C-methyl values were performed by Dr. Franz Pascher, Bonn, Germany.

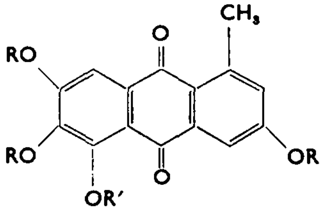
[7] The wax is now under examination.



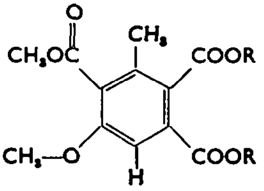
I a, $R = R' = R'' = H$
b, $R = R' = Ac; R'' = H$
c, $R = R' = Ac; R'' = CH_3$
d, $R = R'' = CH_3; R' = H$
e, $R = R'' = CH_3; R' = Ac.$
f, $R = R' = R'' = CH_3$
g, $R = CH_3; R' = R'' = H$
h, $R = R' = CH_3; R'' = H$



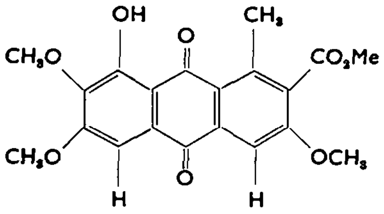
II



III a, $R = R' = Ac$
b, $R = CH_3; R' = H$



IV a, $R = H$
b, $R = CH_3$



V

dissolved in a large volume of boiling MeOH. Ceroalbolinic acid crystallized after concentration of the solution, as dark red prisms. Several crystallizations from MeOH and acetone yielded 8 g of the acid, which begins to decompose at 290° and slowly carbonizes without melting. It gives a positive $FeCl_3$ test, λ_{max} 294, 418 mμ; log ϵ, 4·50 and 3·76, ν_{max} 3500 (hydroxyl groups), 1725 (carboxyl group), 1643 (chelated carbonyl group) 1590 cm^{-1} (C—C double bonds). (Found: C, 58·16; H, 3·04; O, 38·97. C-methyl 4·52. Calc. for $C_{16}H_{10}O_8$: C, 58·19; H, 3·05; O, 38·78; 1 C-methyl 6·60%.)

Ceroalbolinic acid tetraacetate (Ib). To a suspension of Ia (50 mg) in acetic anhydride (5 ml) five drops of perchloric acid were added with mechanical stirring. When the solid was dissolved the mixture was left at room temp for 3 hr. It was poured then in cold water (20 ml) and the crystalline tetraacetate was collected and washed with water. Crystallization from MeOH yielded light yellow prisms (45 mg), m.p. 124–125° (sealed tube); λ_{max} 270, 345 mμ; log ϵ, 4·38, 3·23; ν_{max}; 2900, 1735 (carboxyl group), 1790 (acetyl groups), 1680 and 1590 cm^{-1} (quinonoid chromophore). (Found: C, 57·61; H, 3·85; O, 38·94; acetyl: 34·37. Calc. for $C_{24}H_{18}O_{12}$: C, 57·83; H, 3·64; O, 38·52; for 4 acetyl: 34·50%.)

Ceroalbolinic acid methyl ester tetraacetate (Ic). A solution of Ib (50 mg) in ether was treated with ethereal dizaomethane until a yellow colour persisted. After 1 hr at room temp, the solution was washed with a sat $NaHCO_3$aq and water. The solution was dried over Na_2SO_4 and evaporated to dryness. The analytical sample was obtained by several crystallizations from MeOH, leaflets m.p. 233–243°; yield 50 mg; λ_{max} 262, 330 mμ; log ϵ, 4·38 and 3·64; ν_{max} 1790 (acetyl groups), 1740 (carbonyl of the ester), 1680 (quinonoid carbonyls). (Found: C, 58·57; H, 4·42; O, 37·06. Calc. for $C_{25}H_{20}O_{12}$: C, 58·60; H, 3·93; O, 37·47%.)

Ceroalbolinic acid methyl ester tetramethyl ether (If). Ceroalbolinic acid (50 mg) was dissolved in acetone (100 ml); anhydrous K_2CO_3 (3 g) and $MeSO_4$ (3 ml) were added and the mixture was refluxed for 6·5 hr. Most of the acetone was then distilled off. The residue was diluted with water and extracted with ether. The ethereal solution was first washed with 10% NaOHaq and finally with water, dried over Na_2SO_4 and evaporated to dryness. The solid residue was crystallized from MeOH yielding yellow prisms with m.p. 201–204°; λ_{max} 180, 341 mμ; log ϵ, 4·73 and 3·86; ν_{max} (KBr) 1732 (carbonyl of the ester group) 1665, 1620 and 1578 (quinonoid chromophore). (Found: C, 62·71; H, 5·17; O, 32·37; methoxyl 37·53. Calc. for $C_{21}H_{20}O_8$: C, 62·99; H, 5·04; O, 31·97; for 5 CH_3O, 40·0%.)

Ceroalbolinic acid methyl ester, trimethyl ether (Id). Compound Ia (500 mg) was suspended in ether (100 ml) and treated with ethereal diazomethane. The suspension was stirred until the acid was dissolved. The solution was left at room temp for 2 hr and worked up as in the previous case. Crystallization from $CHCl_3$–MeOH afforded bright leaflets (400 mg), m.p. 245–248°; λ_{max} 281, 312 342, 415 mμ; log ϵ, 4·55; 3·83; 3·55 and 3·68; ν_{max} 1740 (carbonyl of the ester group), 1668 (quinonoid carbonyl group), 1630 (chelated carbonyl group) and 1580 cm^{-1} (C—C double bonds). (Found: C, 62·26; H, 5·09; O, 32·54. Methoxyl groups 29·3; C-methyl 3·62. Calc. for $C_{20}H_{18}O_8$: C, 62·17; H, 4·70; O, 33·13; for 4 CH_3O, 32·1; for 1 C-methyl 3·9%.)

Ceroalbolinic acid methyl ester, trimethyl ether acetate (Ie). To a suspension of Id (100 mg) in a mixture of acetic anhydride (3 ml) and acetic acid (3 ml) 3 drops perchloric acid were added. The solution was left at room temp for 3 hr and worked up as in the previous case. Several crystallizations afforded yellow prisms (60 mg) m.p. 219–222°; λ_{max} 280, 340 mμ; log ϵ, 4·37 and 3·45; ν_{max} 1768 (acetyl band), 1730 (ester group), 1668 and 1580 cm^{-1} (quinonoid chromophore). (Found: C, 61·41; H, 4·66; O, 34·20. Methoxyl: 29·58; C-methyl, 12·59. Calc. for $C_{22}H_{20}O_9$: C, 61·68; H, 4·71; O, 33·61; for 4 CH_3O, 29·1: for 1 C-methyl, 10·3%.)

Reductive acetylation of Ie. Compound Ie (400 mg), was treated with anhydrous sodium acetate (1 g) and powdered Zn (1·2 g) in 7 ml acetic anhydride for 36 hr at 85°. The solvent was evaporated *in vacuo* and the residue diluted with water and collected. Crystallization from $CHCl_3$–MeOH yielded II as yellow prisms, m.p. 249–251°, λ_{max} 277, 349, 368, 386, 407 mμ; log ϵ, 4·95; 3·51; 3·65; 3·62 and 3·55, ν_{max} 1768 (acetyl bands), 1730 (carbonyl ester group), 1625 and 1590 cm^{-1} (C—C double bonds). (Found: C, 60·37; H, 5·08; O, 34·40. Methoxyl groups 23·08. Calc. for $C_{26}H_{26}O_{11}$: C, 60·70; H, 5·09; O, 34·21; for 4 CH_3O, 24·1%.)

Decarboxylation of ceroalbolinic acid (Ia). A solution of Ia (500 mg), in quinoline (20 ml), was refluxed for 3·5 hr with copper chromite (50 mg). The cold solution was diluted with water, filtered, acidified with dil. HCl, and extracted with ether. The ethereal extract was washed with water dried with Na_2SO_4 and evaporated to dryness. The solid residue (240 mg) did not crystallize. The tetraacetate (IIIa) was prepared from 100 mg of the crude decarboxylation product, in 3 ml acetic anhydride, 3 ml acetic acid and 4 drops perchloric acid, at room temp for 1 hr. Crystallization from acetone afforded prisms (40 mg) m.p. 235–237°; λ_{max} 262, 282, 324, 425 mμ; log ϵ, 4·11, 3·64 and 3·11; ν_{max} 1788 (acetyl bands), 1678 (quinonoid carbonyl groups), 1595 cm^{-1} (C—C double bonds). (Found: C, 60·73; H, 4·01; O, 35·17. Acetyl groups 38·87. Calc. for $C_{23}H_{18}O_{10}$: C, 60·79; H, 3·99; O, 35·21; for 4 acetyl groups: 38·0%.)

The trimethyl ether (IIIb) was obtained by methylation of the decarboxylation product (100 mg) with diazomethane. Crystallization from $CHCl_3$–MeOH yielded IIIb as yellow prisms (25 mg) m.p. 221–223°; λ_{max} 280, 310, 413 mμ; log ϵ, 4·21, 3·47, 3·43; ν_{max} 1670 (quinonoid carbonyl band), 1628 (chelated carbonyl band), 1598 (C—C double bonds). (Found: C, 65·71; H, 5·01; O, 29·55. Methoxyl: 28·63. Calc. for $C_{18}H_{16}O_6$: C, 65·85; H, 4·91; O, 29·24; for 3 CH_3O: 28·30%.)

Ceroalbolinic acid trimethyl ether (Ig). Compound Id (100 mg) in ethylene glycol (5 ml) was mixed with KOHaq (5 ml) and MeOH (40 ml). The mixture was refluxed for 30 hr, diluted then with water, acidified with dil HCl and extracted thoroughly with ether. The ethereal solution was extracted with $NaHCO_3$aq. This solution was acidified with dil HCl and extracted with ether. Evaporation of the solvent left a solid residue. Crystallization from $CHCl_3$–MeOH yielded orange crystals (45 mg), m.p. 260–261° (sealed tube); λ_{max} 287, 332, 410 mμ; log ϵ, 4·59, 3·84, 3·63, ν_{max} 3490 (Hydroxyl groups), 1728 (carboxyl group), 1680 (quinonoid carbonyl group), 1640 (chelated carbonyl group), 1590 cm^{-1} (C—C double bonds). (Found: C, 61·26; H, 4·50; O, 34·04. Methoxyl 23·37. Calc. for $C_{19}H_{16}O_8$: C, 61·29; H, 4·33; O, 34·38; for 3 CH_3O: 25·30%.)

Ceroalbolinic acid tetramethyl ether (Ih). A similar saponification of If (100 mg) furnished Ih as yellow plates (from $CHCl_3$–MeOH). m.p. 210–213°, λ_{max} 285, 312 mμ; log ϵ, 4·59, 3·60; ν_{max} 1750, 1712 (carboxyl group); 1670 (quinonoid carbonyl groups) and 1580 cm^{-1} (C—C double bonds). (Found: C, 61·88; H, 4·93; O, 33·13. Methoxyl: 32·09. Calc. for $C_{20}H_{18}O_8$: C, 62·17; H, 4·70; O, 33·38; for CH_3O: 32·10%.)

Cochenillic acid methyl ether mono methyl ester (IVa). Potassium carbonate (1 g) was added to a suspension of Id (400 mg) in acetone (500 ml). The suspension was refluxed for 20 min. A solution of $KMnO_4$ (1 g) in acetone (200 ml) was then added. The mixture was refluxed for 24 hr and during this time 3 g of powdered $KMnO_4$ were added. The precipitate was filtered off and the slight pink solution was concentrated to a small volume. It was diluted with water and a 10% $NaHSO_3$aq was added until the pink colour disappeared. The aqueous solution was extracted with ether. The organic extract was washed with water, dried with Na_2SO_4 and evaporated to dryness. Crystallization from MeOH gave recovered Id (40 mg). The precipitate was suspended in water, acidified with HCl and extracted thoroughly with ether. The ethereal extract was washed with water, dried with Na_2SO_4 and evaporated to dryness. Crystallization from aqueous MeOH afforded IVa (40 mg), m.p. 180–182° (reported,[8] m.p. 178–180°); ν_{max} 3500, 1750 cm^{-1}. (Found: C, 53·73; H, 4·51; O, 41·76. Methoxyl: 23·11; C-methyl 5·59. Calc. for $C_{12}H_{12}O_7$: C, 53·80; H, 4·73; O, 41·80; for 1 CH_3O: 23·51; for 1 C-methyl 4·98%.)

Compound IVb showed m.p. 108–110° plates from acetone–hexane. It did not give depression on a mixed m.p. with an authentic sample,* the IR and NMR spectra were superimposable.

Cochenillic acid methyl ester methyl ether anhydride showed m.p. 149–151° (reported,[8] m.p. 150–151°).

* *Acknowledgments*—The author wishes to thank Dr. J. Romo of this Institute for his generous contribution of time and effort in the writing of this paper.

[8] O. Dimroth, *Chem. Ber.* 43, 1396 (1910).