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VISMIAQUINONE, A Δ^1-ISOPENTENYL SUBSTITUTED ANTHRAQUINONE FROM *VISMIA REICHARDTIANA*

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Key Word Index—*Vismia reichardtiana*; Guttiferae; anthraquinone; Δ^1-isopentenyl side chain; biogenesis.

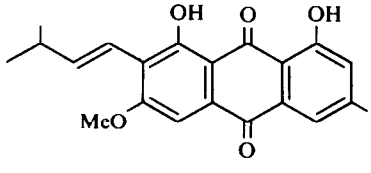
Abstract—1,8-Dihydroxy-7-(Δ^1-isopentenyl)-6-methoxy-3-methylanthraquinone (vismiaquinone) was isolated from the leaves of *Vismia reichardtiana*. Its structure was established through chemical and spectral means. A hypothesis for the biogenesis of the uncommon Δ^1-isopentenyl side chain is presented.

INTRODUCTION

In a recent paper [1], Delle Monache *et al.* described the anthraquinone **1** as a transformation product of vismione A, a tetrahydroanthracene derivative from the fruits of *Vismia baccifera* (L.) Tr. et Pl. subsp. *dealbata* (H.B.K.) Ewan (Guttiferae). We report our findings on compound **1**, for which the name vismiaquinone is proposed, as a genuine constituent of the leaves of *Vismia reichardtiana* (O. Ktze.) Ewan from the north–northeastern part of Brazil. Our material was collected in the vicinity of São Luís, the capital city of the state of Maranhão. The plant, a large shrub, is known under the popular name of 'lacre'. This, as well as several other *Vismia* species, yields a red-orange coloured gum-resin as a stem exudate, which during the 19th century was an article of commerce known as 'American *gummi guttae*'. Used as a substitute for the genuine *gummi guttae* obtained from Asian *Garcinia* species, the product had applications as a constituent of water-colours, a drastic purgative and a topical remedy for skin diseases. The latter use persists in Brazilian folk medicine to this day.

RESULTS AND DISCUSSION

Vismiaquinone (**1**) was isolated from the petrol extract of the dried leaves of *V. reichardtiana* by chromatography (see Experimental, procedure A). That the substance is not an artefact produced during isolation and purification was ascertained by a second extraction with petrol and its separation through direct crystallization under careful manipulation, avoiding all contact with reagents and adsorbents (procedure B).



1 Vismiaquinone

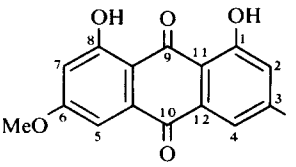
The pure, red crystals melt at 202–204°. Elemental analysis furnishes the molecular formula $C_{21}H_{20}O_5$. IR absorptions suggest the presence of either highly conjugated carbonyl groups or double bonds, as well as an aromatic character. The same is evident from the ^1H NMR spectrum, with broad signals for two aromatic protons suggesting *meta*-coupling.

The presence of phenolic groups is indicated by two signals downfield integrating for one proton each, exchangeable with D_2O. Two phenolic hydroxyl groups were further confirmed by preparation of a diacetate. A singlet corresponding to three protons, at δ 4.02, indicates the presence of one methoxyl group. A doublet integrating for one proton ($J = 16$ Hz) appears at δ 6.6 and is attributed to a *trans*-double bond, corroborated also in the IR by an absorption at 971 cm^{-1}. The position in the ^1H NMR spectrum indicates conjugation with an aromatic ring.

Upfield appears a doublet centred at δ 1.14, corresponding to six protons ($J = 6.5$ Hz), possibly belonging to two geminal methyl groups, coupled with one proton appearing as a multiplet centred at δ 2.48. This suggests the presence of an isopropyl group in the structure. Selective decoupling of the last-mentioned proton caused simplification of the doublet, thus confirming this assumption. Also observed was an alteration in a multiplet located in the region between δ 6.8 and 7.0 and corresponding to one olefinic proton. This indicates that the isopropyl group is α to the double bond in conjugation with an aromatic ring.

The spectrum in the UV/visible region, besides confirming the phenolic nature through a bathochromic shift in alkali, also strongly suggested a quinone structure, possibly a hydroxyanthraquinone. A chrysazine system is suggested by the presence of a strong absorption at 445 nm (log ε 4.2). The quinone nature was confirmed by reduction with sodium borohydride, which caused decolouration, the colour being restored by oxygen. Extraction of **1** from a benzene solution with aqueous alkali produced a colour change to violet, reproducing the classical Bornträger reaction for hydroxyanthraquinones. Also on paper and thin layer chromatograms, the orange colour changed to red when exposed to ammonia vapour.

The evidence in the ^1H NMR spectrum for two aromatic protons with *meta*-coupling shows there is only one additional substituent in one of the aromatic rings. Decoupling of the protons of the methyl group (δ 2.42) resulted in a clear definition ($J = 2$ Hz) of these proton signals (δ 7.03 and 7.56), thus placing the methyl group at C-3 in the anthraquinone skeleton. If the methoxyl is placed at C-6, a common pattern in naturally occurring anthraquinones, then it is apparent that vismiaquinone is simply an isopentenyl derivative of the known 1,8-dihydroxy-6-methoxy-3-methylanthraquinone, parietin **2**. In fact, a comparative analysis of the ^{13}C NMR spectra of vismiaquinone and parietin (**2**) [2] shows complete agreement in the chemical shifts for all the carbon atoms unaffected by this additional substitution (Table 1). The position of the side chain in vismiaquinone can be deduced by comparing the signals of the three aromatic protons in its ^1H NMR spectrum with those of the four aromatic protons of parietin [3, p. 74]. This places the substituent at C-7 (Table 2) and results in structure **1** for vismiaquinone.



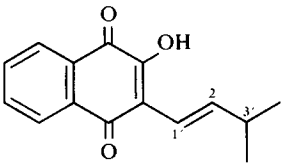
2 Parietin

The rather uncommon conjugated double bond in the isoprenoid side chain received additional support from the ^1H NMR spectrum of vismiaquinone diacetate. This spectrum shows a doublet at δ 6.28 ($J = 16$ Hz), attributable to the proton at C-1′, and a double doublet, centred at δ 6.94 ($J = 16$ and 6.5 Hz) assignable to the proton at C-2′. In the spectrum of vismiaquinone this latter signal is partially hidden by overlap. In the spectrum of the diacetate the aromatic protons suffer a shift downfield, whereas the olefinic protons of the side chain are moved upfield, thus clarifying the assignments. Final proof for the structure of the side chain was obtained by comparison of the ^1H NMR spectrum of vismiaquinone with that of isolapachol (**3**) [5]. Table 3 shows the assignments for the protons relevant to this comparison.

Table 2. ^1H NMR signals of aromatic protons of parietin (**2**) [3, p. 74; see also 4] and vismiaquinone (**1**).

	HC-2	HC-4	HC-5	HC-7	CH_3
Parietin (**2**)	7.08	7.60	7.35	6.67	2.48
Vismiaquinone (**1**)	7.03	7.56	7.34	—	2.42

Measurements in δ values; solvent $CDCl_3$; internal standard TMS.



3 Isolapachol

Prenylation of aromatic substrates is observed rather frequently in the Guttiferae. However, vismiaquinone and vismione A [1] are the first recorded examples of the Δ^1-isopentenyl pattern in isoprenoid substituents in this family. Up to now, this side chain has only been described in three flavones from the wood of *Artocarpus* species (Moraceae), artocarpin [6], norartocarpin [6] and chaplashine [7]. Its ready introduction into the molecule of lawsone by reaction with isovaleraldehyde resulting in isolapachol [5] led us to attempt the same procedure using parietin* as a substrate. However, on applying the same conditions as in the synthesis of isolapachol, parietin was recovered unreacted.

From the biogenetic viewpoint, the structure of the side chain in **1** replacing the much more common dimethylallyl substituent is intriguing. The obvious shift of the double bond into conjugation with the aromatic ring [1, 8] does not explain why such a transposal should not be the rule rather than a rare exception. However, it can be seen that in the few known instances (only five), the substituent in question is attached to an aromatic ring of *polyketide* origin. One may thus assume that in these cases prenylation occurs *before* cyclization of the polyketide chain.† The shift of the double bond can, in fact, be better understood if it takes place as one of the intermediate stages of the biosynthesis, rather than in the final product.

In order to explain the stabilization of intermediates, it has been proposed that during the time the polyketomethylene chains remain attached to the enzyme surface they exist in a partially enolized form [11]. The structure resulting from prenylation of such a substrate would introduce a labile methylenic proton in the side chain, resulting in an anion in which the negative charge is

Table 1. Chemical shifts in the ^{13}C NMR spectrum of vismiaquinone (**1**) and parietin (**2**): correspondence of equivalent carbon atoms

	C-1	C-2	C-3	C-4	C-11	C-12	C-9	C-10	CH_3	OCH_3
Vismiaquinone (**1**)	162.6	124.2	148.1	121.0	113.5	133.0	190.9	182.4	22.1	56.1
Parietin (**2**)	162.5	124.5	148.6	121.3	113.7	133.2	190.8	181.0	22.2	56.1

Measurements in δ values; solvent $CDCl_3$; internal standard TMS.

* Parietin was isolated from the lichen *Theloschistes flavicans* collected at Barra de Maricá, state of Rio de Janeiro.

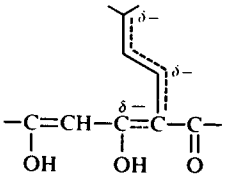
† The possibility of methyl groups being attached to polyketide chains before ring closure in some phenolic compounds has long been admitted [9], and experimental evidence exists that in the lichen *Parmelia tinctorum* introduction of the 'extra' C_1-units into the two constituent phenolcarboxylic acids of the depside atranorin occurs before formation of the aromatic ring [10].

Table 3. ^{1}H NMR signals for protons of the side chain in vismiaquinone (1) and isolapachol (3).

	HC-1′	HC-2′	HC-3′	*gem*-diMe
Vismiaquinone (1)	6.6 (J = 16 Hz)	6.95 (J = 16 and 6.5 Hz)	2.48	1.14 (J = 6.5 Hz)
Isolapachol (3)	6.6 (J = 16 Hz)	7.07 (J = 16 and 6.5 Hz)	2.5	1.14 (J = 6.5 Hz)

Measurements in δ values; solvent $CDCl_3$; internal standard TMS.

distributed over part of the enolic system. A neighbouring carbonyl group would stabilize such a situation (4). Upon ring closure, protonation would take place so that the double bond would become conjugated with the emerging aromatic system.



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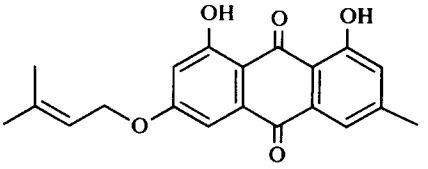

5 Madagascine

Vismiaquinone is the first known anthraquinone with an isoprenoid substituent linked to carbon. In madagascine (5) [12], also from a Guttiferae and also closely related to parietin, the side chain, present in the form of a prenyl ether, was presumably introduced at a late stage in synthesis, so that the allylic structure is retained. The same argument holds for the *Artocarpus* flavones, where a second, but 'normal' isoprenoid substituent is attached *in the cinnamic acid-derived* portion of the molecule.

EXPERIMENTAL

Mps were determined on the Kofler hot stage. UV spectra were obtained in 95% EtOH and IR spectra in KBr. ^{1}H NMR and ^{13}C NMR spectra were measured at 100 MHz and MS at 70 eV.

Extraction. Procedure A. Dried leaves (1.7 kg) of *V. reichardtiana* were exhaustively percolated with petrol (bp 40–80°) and the combined extracts were concd *in vacuo*. The viscous residue was chromatographed on a column of Florisil (Floridin Co.) in petrol. Elution was started with petrol and the polarity of the developing solvent increased gradually. Elution was monitored by TLC on Si gel PF_{254} (Merck). The fraction eluted with petrol–EtOAc (95.5:5), upon concn, separated 1 as orange-red crystals embedded in a yellow oil. The substance was purified by prep. TLC on plates of Si gel PF_{254}, using petrol–EtOAc (9:1). After drying, an orange band was scraped off the plates and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were concd and the product was recrystallized from petrol–EtOAc (19:1), as red needles, mp 202–204°. TLC on Si gel PF_{254} with petrol–EtOAc (9:1) showed one orange spot, R_f 0.38. Yield: 470 mg.

Procedure B. The petrol extract of the leaves, as above, was concd to one fourth of its vol. The conc extract was shaken repeatedly with 95% MeOH. The petrol phase was concd further, again to about one fourth of its vol. After 6 days at room temp. crystals separated. After removal, they were recrystallized from petrol–EtOAc (19:1) yielding a mixture of red and white crystals. Separation of these was accomplished mechanically, under a microscope. The red crystals were identical (mp, mmp, IR, TLC) with 1 of procedure A.

Vismiaquinone (1). Anal.: C, 71.52; H, 5.63; $C_{21}H_{20}O_5$ requires C, 71.58; H, 5.72%. IR ν_{max} cm^{-1}: 3400, 2932, 1667, 1622, 1601, 1558, 1471, 1363, 1220, 1174, 1031, 971, 823, 748. λ_{max}^{EtOH} nm (log ε): 445 (4.2), 295 (4.7), 264 (4.4), 250 (4.4), 224 (4.7). $\lambda_{max}^{EtOH/OH^-}$ nm (log ε): 531 (4.0), 317 (4.2), 278 (4.6), 242 (4.8), 209 (4.7). ^{1}H NMR: δ 1.14 (6 H, *d*, J = 6.5 Hz), 2.42 (3 H, *s*), 2.48 (1 H, *m*), 4.02 (3 H, *s*), 6.6 (1 H, *d*, J = 16 Hz), 6.95 (1 H, *dd*, J = 16 and 6.5 Hz, partial overlap), 7.03 (1 H, *s*), 7.34 (1 H, *s*), 7.56 (1 H, *s*), 12.02 (1 H, *s*), 12.84 (1 H, *s*). MS *m/z* (rel. int.): 352 [M]$^{+\cdot}$ (33), 337 (13), 323 (6), 309 (100), 297 (28), 295 (6), 294 (7), 283 (4), 267 (7), 161 (5), 152 (6). ^{13}C NMR: δ 22.1, 22.46, 33.37, 56.2, 103.1, 110.3, 113.5, 115.68, 119.79, 120.88, 124.19, 131.78, 132.9, 146.48, 148.1, 161.8, 162.2, 162.68, 181.4, 191.0.

Acetylation. 40 mg 1 in 2 ml Ac_2O and 1 ml pyridine were stirred for 3 hr at room temp. and then evapd *in vacuo*. Chromatography on a column of Si gel (2 g) yielded two products. Vismiaquinone monoacetate: Orange needles from hexane–EtOAc (19:1), mp 200–202°. IR ν_{max} cm^{-1}: 3458, 3047, 2937, 1767, 1666, 1627, 1604, 1578, 1483, 1459, 1448, 1350, 1322, 1273, 1203, 1159, 1117, 1104, 982, 902, 763. MS *m/z* (rel. int.): 394 [M]$^+$ (19), 352 (45), 351 (46), 337 (17), 309 (100), 308 (6), 297 (24), 279 (86), 251 (83), 236 (11), 223 (11), 175 (20), 168 (19), 167 (66). Vismiaquinone diacetate: Yellow crystals from EtOAc, mp 197–200°. IR ν_{max} cm^{-1}: 3030, 2920, 1777, 1672, 1658, 1581, 1578, 1455, 1357, 1310, 1250, 1193, 1110, 1086, 897. ^{1}H NMR: δ 1.1 (6 H, *d*, J = 6 Hz), 2.44 (3 H, *s*), 2.49 (1 H, *m*), 2.46 (3 H, *s*), 2.5 (3 H, *s*), 4.04 (3 H, *s*), 6.28 (1 H, *d*, J = 16 Hz), 6.94 (1 H, *dd*, J = 16 and 6.5 Hz), 7.18 (1 H, *s*), 7.66 (1 H, *s*), 7.98 (1 H, *s*). MS *m/z* (rel. int.): 436 [M]$^+$ (4), 394 (33), 352 (51), 351 (63), 337 (14), 309 (100), 297 (25), 283 (4), 279 (2), 267 (5), 251 (2), 236 (1), 223 (2), 165 (3), 150 (1), 149 (8), 133 (2), 119 (1), 105 (3), 91 (4), 77 (2), 69 (12).

Isolapachol (3). Lawsone and isovaleraldehyde were reacted as in ref. [5]. The crude reaction product was chromatographed on a column of Si gel deactivated with 20% water. Brick-red crystals from petrol–EtOAc (4:1), mp 121° (lit. [5] 120°). ^{1}H NMR: δ 1.14 (6 H, *d*, J = 6.5 Hz), 1.65 (1 H, *s*), 2.5 (1 H, *m*), 6.6 (1 H, *d*, J = 16 Hz), 7.07 (1 H, *dd*, J = 16 and 6.5 Hz), 7.7 (2 H, *m*), 8.1 (2 H, *m*). MS *m/z* (rel. int.): 242 [M]$^+$ (22), 227 (100), 213 (9), 200 (18), 199 (48), 190 (20), 174 (15), 171 (10), 162 (35), 146 (10), 105 (65), 104 (20), 76 (55).

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