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MASS SPECTRAL CHARACTERIZATION OF NAPHTHOQUINONES RELATED TO LAPACHOL

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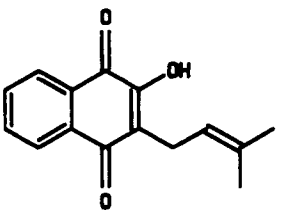
Lapachol [**1**] is a naturally occurring naphthoquinone that has been identified as a constituent of various species of the Bignoniaceae plant family (1) and has been shown to possess oncolytic activity (2). Dehydro-α-lapachone [**2**], 2-isopropylnaphtho[2,3-*b*]furan-4,9-dione [**3**], and dehydro-α-isolapachone [**4**] are three isomeric lapachol derivatives often reported as minor constituents of plant extracts (1,3,4). Their isolation and purification usually require large quantities of plant material and often involve tedious chromatographic separations. In our continuing study of commercial herbal materials marketed as "Taheebo," "Lapacho," "Pau d'Arco," and "Ipe Roxo," we required a simple and reliable method of screening extracts from limited quantities of material. In a previous communication from this laboratory (5), we reported an hplc method of separation and identification of lapachol [**1**] and related naphthoquinones using uv detection. A screening of commercial "Taheebo" products sold in Canada involved these hplc procedures and gc/ms analysis for confirmation of the identity of observed naphthoquinones. However, examination of the published ms data revealed conflicting observations which rendered uncertain the characterization of some of the naturally occurring naphthoquinones of chief interest to us. We here



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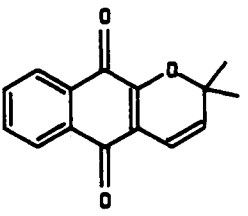

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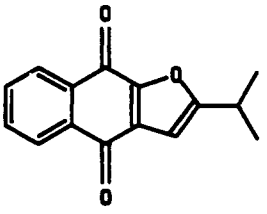

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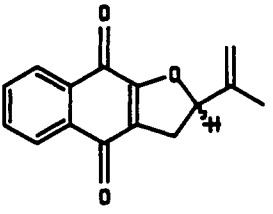

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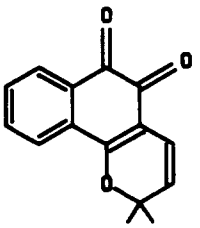

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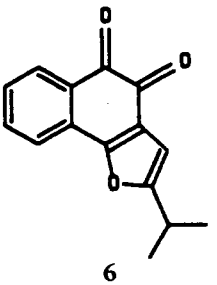

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report a thorough reassessment of the ms behavior of these lapachol-related compounds.

The mass spectra of isomeric naphthoquinones **2-6** were recorded, and for each compound, excluding dehydro-β-lapachone [**5**], identical spectra were obtained when using either direct-probe or gc inlet methods. Table 1 presents the data recorded for the major fragments observed. All compounds gave distinct molecular ions at *m/z* 240 and showed fragmentation patterns that can be accounted for in terms of well-known decay processes (6-8). In agreement with other reports (7,8), compounds **5** and **6** failed to show associated $[M+2]^+$ ion peaks characteristic of most 1,2-naphthoquinones (9). Hence, due to the similarity of fragmentation patterns, only intensity variations can be used here to differentiate between the various isomers.

The mass spectrum of dehydro-α-lapachone [**2**] has been published previously on two separate occasions (7,8), and, although similar fragmentation patterns were reported, the relative intensities of the various fragments were markedly different. The spectrum of **2** recorded in this study agrees essentially with the one reported by Elwood *et al.* (7) and is characterized by a high $[M-15]^+/[M]^+$ ratio (>5) when compared with all four other isomers. The spectrum of **3**, which also features its base peak at *m/z* 225, is distinguished from **2** by the lower $[M-15]^+/[M]^+$ and $[M-43]^+/[M]^+$ ratios, both resulting from the enhanced relative intensity of the molecular ion peak. The isomeric furano-1,2-naphthoquinone [**6**], on the other hand, presents a more easily distinguishable spectrum featuring the base peak at *m/z* 197, while showing a much lesser import of the fragment at *m/z* 225. Dehydro-α-isolapachone [**4**] also gives the base peak at *m/z* 197 along with a prominent fragment at *m/z* 212, which appears to be characteristic of this compound.

A distinctive spectrum of dehydro-β-lapachone [**5**] was obtained only by the direct-probe insertion method because its relative instability leads to its complete conversion to the more stable, isomeric dehydro-α-lapachone [**2**] when the gc inlet method was used. Its spectrum is characterized by prominent peaks at *m/z* 197 and 225 (base peak) and shows the most intense molecular ion peak of all isomers studied.

The data presented here clearly show that unambiguous identification of the

TABLE 1. Mass Spectral Data for Dehydro-α-lapachone [**2**], 2-Isopropylnaphtho[2,3-*b*]furan-4,9-dione [**3**], Dehydro-α-isolapachone [**4**], Dehydro-β-lapachone [**5**], and 2-Isopropylnaphtho[1,2-*b*]furan-4,5-dione [**6**][a]

m/z	Compounds				
	2	**3**	**4**	**5**[b]	**6**
240	17.9 (1.00)	44.9 (1.00)	26.8 (1.00)	67.3 (1.00)	48.1 (1.00)
225	100.0 (5.58)	100.0 (2.23)	41.5 (1.55)	100.0 (1.49)	19.0 (0.39)
212	4.3 (0.24)	3.8 (0.09)	99.7 (3.72)	22.8 (0.34)	5.1 (0.11)
197	34.6 (1.93)	26.6 (0.59)	100.0 (3.73)	96.3 (1.43)	100.0 (2.08)
183	3.1 (0.17)	2.2 (0.05)	16.3 (0.61)	8.6 (0.13)	<1.0 (—)
169	4.9 (0.28)	5.1 (0.11)	19.3 (0.72)	13.4 (0.20)	2.2 (0.05)
141	9.3 (0.51)	13.4 (0.30)	20.9 (0.78)	21.4 (0.32)	11.8 (0.25)
133	9.8 (0.52)	5.1 (0.11)	24.4 (0.91)	31.0 (0.46)	<1.0 (—)
115	14.2 (0.79)	17.1 (0.38)	30.3 (0.48)	32.1 (0.48)	15.2 (0.32)
105	17.3 (0.97)	17.2 (0.38)	50.7 (1.89)	39.1 (0.58)	12.7 (0.26)
76	16.4 (0.92)	11.5 (0.26)	63.2 (2.36)	44.3 (0.66)	6.1 (0.13)

[a]Relative intensity (%) (peak heights relative to $[M]^{\cdot+} = 1.00$).
[b]Spectrum recorded using direct exposure probe.

isomeric naphthoquinones **2-6** can be accomplished by ms simply by determining the intensity variations of the $[M]^+$, $[M-15]^+$, $[M-28]^+$, and $[M-43]^+$ ions, little diagnostic information being provided by the lower mass fragments. This technique, employed in conjunction with hplc or gc separation techniques, allows unequivocal identification of the naphthoquinone constituents of plant extracts.

EXPERIMENTAL

METHODS.—^{1}H-nmr spectra were recorded on a Bruker spectrometer. Samples were dissolved in $CDCl_3$, and TMS was used as an internal standard. Data are expressed in δ ppm. Mass spectra were recorded on a Finnigan MAT 4610B mass spectrometer operating in the electron-impact mode. The operating conditions were: 150° ionizer temperature, 43 eV ionizing voltage, 300 μA emission current, and 900-1000 V range for electron multiplier voltage. Samples were introduced via unheated, direct exposure probes (DEP) or gc inlets.

Gc was performed on a Finnigan MAT 9611 in the split mode (30:1) with a J&W fused-silica capillary column (DB-5, 15 m, 0.25 mm I.D., 0.25 μm coating thickness). Gc conditions were: 250° injection and interface temperature; He carrier gas, 48.3 kpascal column pressure, oven temperature: 100° for 1 min, 100°-250° at 15°/min, 250° for 4 min.

MATERIALS.—Dehydro-α-lapachone [**2**] (10), β-isopropylfurano-1,4-naphthoquinone [**3**] (11), β-isopropylfurano-1,2-naphthoquinone [**6**] (11), and dehydro-β-lapachone [**5**] (12) were all prepared by standard published procedures.

Dehydro-α-isolapachone [**4**] was obtained from a plant extract in the following manner: the light petroleum ether extract of the dried plant material was evaporated to dryness and taken up in Et_2O. Lapachol [**1**] was removed by extraction with 2 N Na_2CO_3, and the resulting Et_2O layer was dried over anhydrous $MgSO_4$. The major component was separated by preparative hplc and identified as dehydro-α-isolapachone [**4**] on the basis of its spectroscopic properties (3,4) and comparison with an authentic sample prepared synthetically (13). ^{1}H-nmr ($CDCl_3$) 1.83 (s, 3H, CH_3), 3.02 (dd, 1H, CH_2), 3.38 (dd, 1H, CH_2), 4.98 (s, 1H, ), 5.12 (s, 1H, ), 5.40 (dd, 1H, ), 7.68 and 8.07 (multiplets, 4H, aromatics); uv (λ max, EtOH) 250, 286, 340 nm; ms *m/z* 240 (M^+).

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