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# BIOACTIVE FURANONAPHTHOQUINONES FROM *CRESCENTIA CUJETE*

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ABSTRACT.—Bioassay-directed fractionation of the MeCOEt extract of *Crescentia cujete* (Bignonaceae) resulted in the isolation of (2*S*,3*S*)-3-hydroxy-5,6-dimethoxydehydroiso-α-lapachone [**1**], (2*R*)-5,6-dimethoxydehydroiso-α-lapachone [**2**], (2*R*)-5-methoxydehydroiso-α-lapachone [**3**], 2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione [**4**], 5-hydroxy-2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione [**5**], 2-isopropenylnaphtho[2,3-*b*]furan-4,9-dione [**6**], and 5-hydroxydehydroiso-α-lapachone [**7**]. Compounds **1–3** are new, and all compounds are bioactive, showing selective activity towards DNA-repair-deficient yeast mutants. The isolation, structure elucidation, and biological activities of these compounds are reported.

In our continuing effort to isolate novel anticancer agents from natural sources, a simple and facile bioassay is being used to monitor the isolation of bioactive compounds. The assay utilized is based on the differential response of DNA-repair-deficient and repair-proficient yeast strains to the test sample. A genetically engineered yeast strain that lacks the recombination pathway associated with repair of double-strand breaks and meiotic recombination, known as rad 52, is the basis of our assay. Details concerning the assay have been previously reported (1,2).

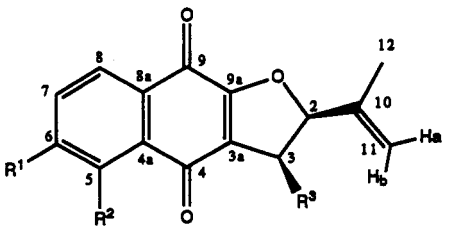
As part of an ongoing collaborative effort, an MeCOEt extract of *Crescentia cujete* L. (Bignoniaceae) was found to show selective activity against the rad 52 yeast strain RS322YK. Bioassay-directed fractionation of this extract yielded seven furanonaphthoquinones **1–7**, three of which are new and five of which have not been previously reported form this genus. The isolation, characterization, and biological activity of compounds **1–7** are herein reported.

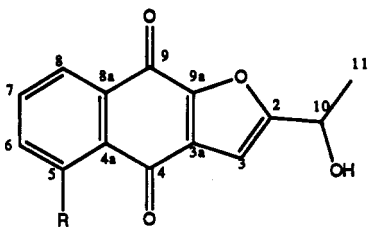
## RESULTS AND DISCUSSION

A crude extract of *C. cujete* was obtained by soaking pulverized wood chips first in hexanes and then in cold MeCOEt. Solvent-solvent partitioning of the dried MeCOEt extract with hexane/80% aqueous MeOH was carried out, followed by dilution of the 80% aqueous MeOH fraction to 60% aqueous MeOH and thorough extraction of this fraction with $CHCl_3$. Activity was concentrated in the $CHCl_3$ extract.

Flash chromatography of the dried $CHCl_3$ extract yielded four active fractions. These were further purified by additional cc, normal and reversed-phase preparative tlc, and reversed-phase hplc, yielding the seven bioactive compounds **1–7**. All of the compounds were obtained as brightly colored solids, and uv, ir, and $^1$H-nmr spectra suggested they were furanonaphthoquinones with various functionalities.

The hreims of **1** indicated that it had the molecular formula $C_{17}H_{16}O_6$. Its uv and ir spectra were consistent with those for naphthoquinones (3), and comparison of the $^1$H-nmr spectrum of **1** with those of known isolapachones (4–8) indicated that **1** belonged to this class. Two 3H singlets at δ 3.92 and 3.99 ppm implied the presence of two MeO groups, and two *ortho*-coupled doublets for aromatic protons at δ 7.12 and 7.96 ppm indicated that the MeO groups must be assigned to the 5,6 or to the 7,8 positions.





**1** $R^1=R^2=OMe$, $R^3=OH$
**2** $R^1=R^2=OMe$, $R^3=H$
**3** $R^1=R^3=H$, $R^2=OMe$
**6** $R^1=R^2=R^3=H$ Undefined stereochemistry
**7** $R^1=R^3=H$, $R^2=OH$ Undefined stereochemistry
**11** $R^1=R^2=OMe$, $R^3=OCO(CF_3)(OMe)C_6H_5$

**4** R=H
**5** R=OH

Because of the low three-bond C-4 to H-3 coupling constant $^3J$ (C,H)=1.5 Hz, this correlation was not seen in a typical HMBC nmr experiment, and selective INEPT methods (4,9) were used to locate the MeO groups. Thus, irradiation of H-8 at δ 7.96 ppm under selective INEPT conditions (4 Hz) enhanced the signal for C-9 at δ 177.0 ppm, while irradiation of H-3 at δ 5.37 ppm (1.5 Hz) enhanced the signal of C-4 at δ 182.6 ppm; these data clearly showed that the MeO groups are at the 5,6 positions.

The remaining three carbons of **1** could be assigned to an isopropenyl group and an OH group, which must be located at the 2 and 3 positions, respectively, on the basis of the chemical shifts of H-2 and H-3 and HMBC nmr correlations. Conflicting $^1$H-nmr assignments for the terminal olefin protons ($H_a$-11 and $H_b$-11) in the literature (4,5) prompted the use of nOe difference nmr experiments to settle the discrepancy. Irradiation of the signal at δ 1.79 ppm due to the Me group enhanced the signal of $H_a$-11 at δ 5.00 ppm, and $H_b$-11 at δ 5.15 ppm was unaffected; while an nOe on the Me group was seen upon irradiation of $H_a$-11, whereas irradiation of $H_b$-11 showed no significant enhancement of the Me group signal. Definitive assignments are listed in Table 1.

The relative stereochemistry of the isopropenyl and OH groups must be cis on the basis of the observed $J_{2,3}$ value of 4.5 Hz (4). Compounds similar to **1** have been isolated as mixtures of enantiomers (4). In order to determine if both enantiomers of **1** were

TABLE 1. $^1$H nmr Chemical Shifts (400 mHz) of Isolapachones **1–3** in $CDCl_3$.[a]

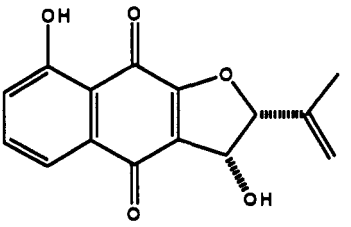
| Proton | Compound | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| H-2 | 5.08 (d, 4.6) | 5.38 (dd, 10.6, 9.2) | 5.37 (dd, 10.4, 9.1) |
| H-3α | 5.37 (d, 4.6) | 3.30 (dd, 17.4, 10.3) | 3.32 (dd, 17.5, 6.7) |
| H-3β | | 2.99 (dd, 17.4, 8.7) | 2.99 (dd, 17.5, 8.8) |
| H-6 | — | — | 7.31 (dd, 8.4, 0.98) |
| H-7 | 7.12 (d, 8.6) | 7.10 (d, 8.6) | 7.62 (dd, 8.5, 7.5) |
| H-8 | 7.96 (d, 8.6) | 7.94 (d, 8.6) | 7.66 (dd, 7.7, 1.1) |
| $H_a$-11 | 5.00 (d, 0.5) | 4.98 (d, 0.9) | 4.98 (d, 0.9) |
| $H_b$-11 | 5.15 (d, 0.9) | 5.11 (d, 0.9) | 5.11 (d, 0.9) |
| H-12 | 1.79 (s) | 1.79 (s) | 1.79 (s) |
| 5-OMe | 3.92 (s) | 3.91 (s) | 3.99 (s) |
| 6-OMe | 3.99 (s) | 3.97 (s) | — |

[a]Chemical shifts (relative to TMS) are in ppm and coupling constants (in parentheses) in Hz.

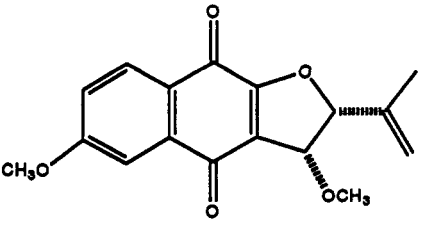
present, its (+)-α-methoxy-α-trifluoromethylphenylacetate (MTPA) ester derivative was prepared (10). The $^1$H-nmr spectrum of this ester showed the H-3 doublet shifted downfield from δ 5.37 to 6.60 ppm, and it appeared as a single resonance. The new MeO group (from the MTPA ester) appeared as a single resonance at δ 3.54 ppm. The fact that both of these signals appeared as single resonances indicated the presence of a single enantiomer. Determination of the absolute stereochemistry was made by comparison of the cd spectrum of **1** with those of known compounds **8** (11) and **9** (5). The cd spectrum of **1** appears to be the mirror image of those reported for **8** and **9**. Since the configurations at C-2 and C-3 have been established as *R,R* for **8** and **9**, it follows that the opposite configurations exist in **1**; this is supported by the fact that the specific rotations of **8** and **9** are opposite in sign to that for **1**. Hence compound **1** is assigned as (2*S*,3*S*)-3-hydroxy-5,6-dimethoxydehydroiso-α-lapachone. Complete $^1$H- and $^{13}$C-nmr assignments are given in Tables 1 and 2, respectively.

Compound **2** was assigned the composition $C_{17}H_{16}O_5$ by hreims, and its $^1$H-nmr spectrum indicated the presence of two MeO groups and an isopropenyl group. The major difference between the $^1$H-nmr spectra of **1** and **2** was at C-3, where **2** showed two doublets of doublets at δ 2.99 and 3.30 ppm, consistent with the presence of a 2,3-dihydrofuran ring. Assignments for $H_a$-11 and $H_b$-11 were based on nOe difference nmr experiments, as for compound **1**. Irradiation of $H_a$-11 at δ 4.98 ppm enhanced the Me group signal and had no effect on the H-3 protons. Irradiation of $H_b$-11 at δ 5.11 ppm also enhanced the Me group signal but to a lesser degree than when $H_a$-11 was irradiated. More importantly, irradiation at $H_b$-11 showed enhancement of both H-3 protons at δ 2.99 and 3.30 ppm. Irradiation of the Me group protons at δ 1.79 ppm showed enhancement of $H_a$-11 and had no effect on $H_b$-11. Irradiation of the signal at δ 1.79 ppm also showed enhancement of the H-3 signal at δ 2.79 ppm, defining it as the β proton. Placement of the MeO groups at C-5 and C-6 was again achieved by selective INEPT experiments: irradiation of H-8 at δ 7.94 ppm (4 Hz) enhanced C-9 at δ 176.8 ppm, while irradiation of $H_a$-3 at δ 2.99 ppm (1.5 Hz) enhanced the signal for C-4 at δ 182.1 ppm. Complete $^1$H- and $^{13}$C-nmr assignments are given in Tables 1 and 2, respectively.

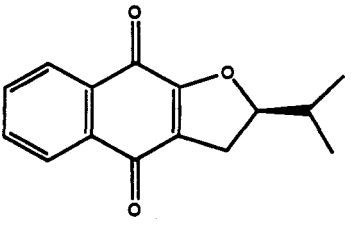
The absolute stereochemistry at C-2 of **2** was determined by comparison of its cd spectrum with that for compound **10** (11). The cd spectrum of **2**, although shifted slightly to a longer wavelength, is nearly identical to that for **10**, each characterized by




**8**




**9**




**10**

TABLE 2. $^{13}$C-nmr Chemical Shifts (100.57 mHz) of Isolapchones **1–3** in $CDCl_3$.[a]

| Carbon | Compound | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| C-2 | 95.1 | 88.5 | 88.3 |
| C-3 | 75.4 | 32.4 | 32.5 |
| C-3a | 125.0 | 125.5 | 126.0 |
| C-4 | 182.5 | 182.1 | 182.5 |
| C-4a | 124.1 | 126.1 | 134.24 |
| C-5 | 149.5 | 149.5 | 159.7 |
| C-6 | 160.1 | 160.0 | 119.2 |
| C-7 | 114.5 | 114.3 | 134.18 |
| C-8 | 125.2 | 124.8 | 119.6 |
| C-8a | 125.9 | 125.8 | 120.0 |
| C-9 | 177.0 | 176.8 | 177.7 |
| C-9a | 159.7 | 159.1 | 157.9 |
| C-10 | 139.4 | 141.9 | 142.0 |
| C-11 | 113.9 | 113.9 | 113.8 |
| C-12 | 17.2 | 17.0 | 17.0 |
| 5-OMe | 56.4 | 56.4 | 56.7 |
| 6-OMe | 61.3 | 61.4 | — |

[a]In ppm from TMS. Assignments based on HMQC (140 Hz) and HMBC (3 and 8 Hz) correlations.

a negative first Cotton effect. Thus, it could be concluded that **2** is (2*R*)-5,6-dimethoxydehydroiso-α-lapachone.

The hreims of **3** indicated that it had the molecular formula $C_{16}H_{14}O_4$, and its $^1$H-nmr spectrum showed the presence of an isopropenyl group as well as the multiplets for the H-3 protons (δ 2.99 and 3.32 ppm) of the furan moiety as seen for compound **2**. The $^1$H-nmr spectrum indicated the presence of a single MeO group; an ABX pattern and the coupling constants for the aromatic proton signals (δ 7.66, 7.62, and 7.71 ppm) suggested that it be placed at either C-5 or C-8. Selective INEPT experiments assigned the MeO group to C-5: irradiation of H-8 at δ 7.66 ppm (4 Hz) enhanced C-9 at δ 177.7 ppm, while irradiation of $H_a$-3 at δ 2.99 ppm (1.5 Hz) enhanced the signal for C-4 at δ 182.5 ppm. Complete $^1$H- and $^{13}$C-nmr assignments are given in Tables 1 and 2, respectively. The absolute stereochemistry of **3** was based on the fact that its cd spectrum is nearly identical to that for compound **2**; the same reasoning used for **2** applied here as well. Thus, **3** is (2*R*)-5-methoxydehydroiso-α-lapachone.

The structures of compounds **4–7** were determined by comparison of $^1$H-nmr, ir, mp, and ms data with literature values (3–7).

The biological activity data for compounds **1–7** in our mechanism-based yeast mutant bioassays are given in Table 3. All seven compounds showed moderate but selective activity against the repair-deficient rad 52 yeast strain, indicating that they act as DNA-damaging agents. Compounds **1–5** were also tested for cytotoxicity against Vero cells; the highest potencies were shown by compounds **4** and **5**, both of which have an OH in their side chain. The activity of **4** against Vero cells is about an order of magnitude greater than its previously reported cytotoxicity to KB cells (8).

This work demonstrates the ability of the yeast bioassay to lead to the isolation of compounds that have significant activity in other established bioassays. The fact that the isolated compounds are all planar suggests that intercalation into DNA may be involved in the mechanism of DNA damage.

TABLE 3. Bioactivity of Furanonaphthoquinones **1–7**.

| Compound | Yeast Strain | | Cytotoxicity to Vero cells |
|---|---|---|---|
| | RS 322 YK (rad 52)[a] | RS 188 N (RAD $^+$)[a] | $IC_{50}$, μg/ml |
| **1** | 47 | >1000 | 3.7 |
| **2** | 33 | 120 | 4.7 |
| **3** | 48 | 280 | 4.3 |
| **4** | 14 | 180 | 0.41 |
| **5** | 3 | 10 | 0.21 |
| **6** | 60 | 130 | NT[c] |
| **7** | 80 | 140 | NT[c] |
| Camptothecin[b] | 0.6 | 110 | 0.054 |

[a]Results are expressed as $IC_{12}$ values in μg/ml (concentration required to produce an inhibition zone of 12 mm around a 100 μl well in the yeast strain).

[b]Standard reference compound.

[c]Not tested.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—These were identical to those reported by Gunatilaka *et al.* (2). The cytotoxicity data were obtained with a 72-h exposure to Vero cells using the endpoint of XTT staining.

PLANT MATERIAL.—The plant material (PR-43072) was collected in Panama in 1974, and a voucher specimen has been deposited at the Herbarium of the National Arboretum, Agricultural Research Service, USDA, Washington, D.C.

ISOLATION OF FURANONAPHTHOQUINONES.—Dried wood chips of *C. cujete* (3.2 kg) were ground into mulch, extracted sequentially with cold hexane, MeCOEt, MeOH, and finally $H_2O$. The bioactive constitutents were concentrated in the MeCOEt extract (12 g dried). The crude MeCOEt extract (12 g) was partitioned between hexane and 80% aqueous MeOH. $H_2O$ was added to the aqueous MeOH fraction until a 60% aqueous MeOH mixture was achieved. This was extracted thoroughly with $CHCl_3$. The $CHCl_3$ extract was dried under vacuum to yield 6.5 g of active material. This fraction (6.5 g) was loaded onto a Si gel column [15 cm×50 mm (600 ml column volume)] and compounds were eluted with a gradient of EtOAc in hexane (30–80%), collecting 50-ml fractions.

Fractions 2–5 (I) eluted as a single yellow band in 30% EtOAc; they were combined and dried (38 mg). Fractions 26–33 (II), eluted with 50% EtOAc, came off as an orange band (179 mg). Fractions 43–63 (III) were combined based on similar tlc, then dried (920 mg). Finally, a large orange band eluted with 80% EtOAc into fractions 64–79 (IV) (274 mg). Each of these four fractions showed bioactivity, and each was further purified as described below.

Combined fraction I (38 mg) was applied to two 1000-μm reversed-phase preparative tlc plates (20×20 cm) and eluted with 80% aqueous MeOH. The two major yellow bands at $R_f$ 0.35 and 0.45 were collected, extracted with $Me_2CO$, and dried to yield 3 mg of **6** (6) and 1.5 mg of **7** (7).

Fraction II (179 mg) was loaded onto a Si gel column 15 cm×18 mm, and eluted with a gradient of $Me_2CO$ in $CH_2Cl_2$ (0–5%), collecting 10-ml fractions. Fractions 11–14, eluting with 1% $Me_2CO$ were combined and dried (28 mg). This was applied to two 1000-μm reversed-phase preparative tlc plates, eluted with 80% aqueous MeOH, and the major yellow band was collected and extracted with $Me_2CO$. The resulting material was recrystallized from EtOH to yield 13 mg of pure compound **2**. Fractions 17–19 were combined and recrystallized from EtOH to yield 31 mg of the known isolapachone **5** (4).

Combined fraction III (920 mg) was subjected to cc on Si gel (50 g) and eluted with 5% $Me_2CO$ in $CH_2Cl_2$; 22 fractions of 25 ml each were collected. Fractions 3–6 were combined and dried to yield 40 mg of material which was applied to two 1000-μm reversed-phase preparative tlc plates and eluted with 70% aqueous MeOH. The major yellow band at $R_f$ 0.4 was collected, extracted with $Me_2CO$, and dried to yield, after recrystallizing from EtOH, 24 mg of pure **3**.

The remaining fraction IV (274 mg) was applied to 15 g Si gel in a 20 mm wide column (height 15 cm, column volume 50 ml) and eluted with a gradient of $Me_2CO$ in $CH_2Cl_2$ (0–7%), collecting 10-ml fractions. A single orange band eluted into fractions 16–18, which were combined, and after reversed-phase

preparative tlc on one 1000-μm plate and final purification by reversed-phase hplc, 3 mg of the known furanonaphthoquinone **4** (8) was obtained.

Fractions 30–35 were combined and dried to yield 20 mg of material which was applied to one reversed-phase preparative tlc plate and eluted with 80% aqueous MeOH. The major yellow band at $R_f$ 0.35 was collected, washed with $Me_2CO$, and dried. Recrystallization from EtOH yielded 13 mg of pure compound **1**.

*(2S,3S)-3-Hydroxy-5,6-dimethoxydehydroiso-α-lapachone* [**1**].—Yellow needles: mp 140–142°; [α]D +17.59 (1.86, $CHCl_3$); cd (MeCN) θ (nm) −20,000 (212), +7000 (245), +2000 (270), +2200 (283), −11,000 (302), 0 (327), +1200 (375), 0 (485); uv λ max (log ε) in MeOH 270 (4.10), 300 (3.98), 360 (3.59), 4.06 (3.32); ir ν max (KBr) 3380, 1680, 1655, 1640, 1570, 1480, 1270, 1200, 1050, 970, 845 $cm^{-1}$; $^1H$ nmr see Table 1; $^{13}C$-nmr see Table 2; hreims $[M]^+$ 316.0947 ($C_{17}H_{16}O_6$ requires 316.0945), 301 (20), 287 (18), 272 (26), 255 (18), 233 (20), 160 (15), 149 (35), 104 (22), 76 (33), 55 (98).

*(2R)-5,6-Dimethoxydehydroiso-α-lapachone* [**2**].—Yellow-orange powder: mp 105–106°; [α]D −11.97 (0.137, $CHCl_3$); cd (MeOH) θ (nm) −10,700 (215), −3829 (245), 0 (258), +2780 (270), −2000 (283), −7430 (302), −1560 (327), −840 (485); uv λ max (log ε) in MeOH 245 (4.09), 270 (3.99), 300 (3.79), 350 (3.42); ir ν max (KBr) 1670, 1650, 1630, 1560, 1470, 1270, 1233, 1210, 1060, 975, 940, 895, 840, 750 $cm^{-1}$; $^1H$ nmr see Table 1; $^{13}C$ nmr: see Table 2; hreims $[M]^+$ 300.1016 ($C_{17}H_{16}O_5$ requires 300.0998), 285 (38), 267 (14), 257 (55), 245 (39), 229 (15), 217 (60), 165 (35), 149 (60), 129 (40), 115 (37), 104 (39), 76 (41), 65 (32), 57 (39).

*(2R)-5-Methoxydehydroiso-α-lapachone* [**3**].—Yellow needles: mp 123°; [α]D −17.44 (0.387, $CHCl_3$); cd (MeOH) θ (nm) −6200 (224), −2000 (250), +1500 (263), 0 (270), −4900 (290), −500 (315), +1100 (420), 0 (500); uv λ max (log ε) in MeOH 243 (4.19), 280 (3.98), 324 (3.40), 387 (3.40); ir ν max (KBr) 2910, 1675, 1640, 1580, 1470, 1440, 1270, 1220, 1070, 980, 870, 750 $cm^{-1}$; $^1H$ nmr see Table 1; $^{13}C$ nmr see Table 2; hreims $[M]^+$ 270.0894 ($C_{16}H_{14}O_4$ requires 270.0892), 255 (50), 242 (25), 227 (82), 215 (15), 187 (30), 163 (32), 135 (38), 134 (36), 115 (25), 104 (40), 76 (92), 55 (42).

*α-Methoxy-α-trifluoromethylphenyl acetate (MTPA) ester of 1* [**11**].—Compound **1** (2.7 mg) was coupled with (*R*)-(+)-α-methoxy-α-trifluoromethylphenyl acetic acid using DCC and DMAP under the usual conditions to give the MTPA ester **11** as a yellow semi-solid in quantitative yield: $^1H$ nmr ($CDCl_3$) δ 1.86 (3H, s, Me-12), 3.54 (3H, d, $J$=0.8, MTPA OMe), 3.85 (3H, s, 5-OMe), 5.02 (1H, br s, $H_a$-11), 5.15 (1H, br s, $H_b$-11), 5.30 (1H, br s, H-2), 6.60 (1H, d, $J$=3, H-3), 7.12 (1H, d, $J$=8.4, H-7), 7.41 (3H, m), 7.58 (2H, m), 7.98 (1H, d, $J$=8.5, H-8).

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## LITERATURE CITED

1. R.K. Johnson, H.F. Bartus, G.A. Hoffman, J.O. Bartus, S.-M. Mong, L.F. Faucette, F.L. McCabe, J.A. Chan, and C.K. Mirabelli, in: "*In Vitro* and *In Vivo* Models for Detection of New Antitumor Drugs." Ed. by L.J. Hanka, T. Kondo, and R.J. White, Organizing Committee of the 14th International Congress of Chemotherapy, Kyoto, 1986, pp. 15–26.
2. A.A.L. Gunatilaka, G. Samaranayake, D.G.I. Kingston, G. Hoffmann, and R.K. Johnson, *J. Nat. Prod.*, **55**, 1648 (1992).
3. R.H. Thompson, "Naturally Occurring Quinones III. Recent Advances," Chapman and Hall, London, 1987, pp. 125–343.
4. H. Wagner, B. Kreher, H. Lotter, M.O. Hamburger, and G.A. Cordell, *Helv. Chim. Acta*, **72**, 667 (1989).
5. K. Inoue, C.C. Chen, H. Inouye, and K. Kuriyama, *J. Chem. Soc., Perkin Trans. 1*, 2764 (1981).
6. W. Sanderman, M.H. Shimatupang, and W. Wendeborn, *Naturwissenschaften*, **55**, 38, (1968).
7. C.C. Chen, *Hua Hsueh*, **41**, 9 (1983).
8. M.M. Rao and D.G.I. Kingston, *J. Nat. Prod.*, **45**, 600 (1982).
9. A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
10. J.A. Dale and H.S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
11. S. Ueda, K. Inoue, Y. Shiobara, I. Kimura, and H. Inouye, *Planta Med.*, **40**, 168 (1980).

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