ELECANACIN, A NOVEL NEW NAPHTHOQUINONE FROM THE BULB OF *ELEUTHERINE AMERICANA*

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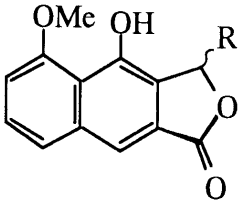
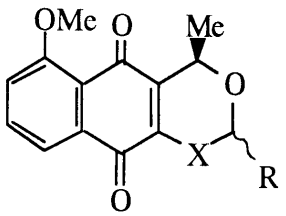
A novel new naphthoquinone called elecanacin (**9**) and a new naphthalene called isoeleutherol (**10**) were isolated from the bulb of *Eleutherine americana* MERR. *et* HEYNE (Iridaceae), together with two known naphthoquinones (**2**and **3**). The structures were determined by spectroscopic methods including the 2D-NMR techniques. Eleutherin (**2**) showed interesting inhibitory activity against human topoisomerase II, and isoeleutherin (**3**) and isoeleutherol (**10**) showed inhibitory activity against HIV.

KEY WORDS *Eleutherine americana*; Iridaceae; elecanacin; isoeleutherol; human topoisomerase II inhibitor; anti-HIV

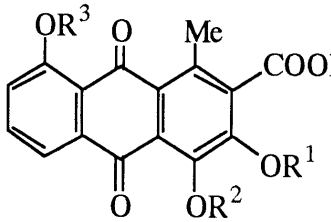
Eleutherine americana MERR. *et* HEYNE (Iridaceae) is widely cultivated in Hainan Island, China, as a folk medicine to treat coronary disorders. Eleutherol (**1**), eleutherin (**2**), isoeleutherin (**3**), hongconin (**4**), and four anthraquinone derivatives (**5**~**8**) were isolated[1] from the bulb of *E. americana*.



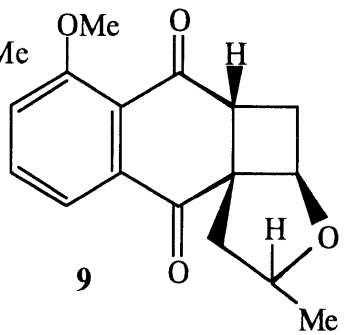
1: R = β-Me
10: R = α-Me



2: R = β-Me ; X = CH_2
3: R = α-Me ; X = CH_2
4: R = α-Me ; X = CO



5: R^1 = Me, R^2 = R^3 = H
6: R^1 = R^3 = H, R^2 = Me
7: R^1 = R^2 = R^3 = Me
8: R^1 = R^2 = Me, R^3 = H



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In our continuing search for bioactive naphtoquinone constituents from the plants of Iridaceae, we have isolated a new novel naphtoquinone called elecanacin (**9**) and a new naphthalene called isoeleutherol (**10**), together with **2** and **3**, from the bulb of *E. americana*, which was collected on Java Island, Indonesia. This communication deals with the structural elucidation of elecanacin (**9**) and isoeleutherol (**10**), and the inhibitory activities

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of **2**, **3**, **9**, and **10** against human topoisomerase II (TOPO II) and human immunodeficiency virus (HIV).

The ethanol extract (14.6 g) of the bulb (dried weight, 5 kg) of *E. americana* was subjected to silica gel column chromatography followed by HPLC (Asahipak ODP 90) to yield eleutherin (**2**, 32 mg), isoeleutherin (**3**, 25 mg), elecanacin (**9**, 16 mg), and isoeleutherol (**10**, 12 mg).

Elecanacin (**9**), in the form of brown pillars, mp 196 ~197°C (Acetone), $[\alpha]_D$ + 20.7° ($CHCl_3$), was established as $C_{16}H_{16}O_4$ by HR-MS and elemental analysis. The ^1H- and ^{13}C-NMR studies of **9** including ^1H-^1H COSY and HMQC spectra suggested the structures [A, B, and C] shown in Fig. 1.

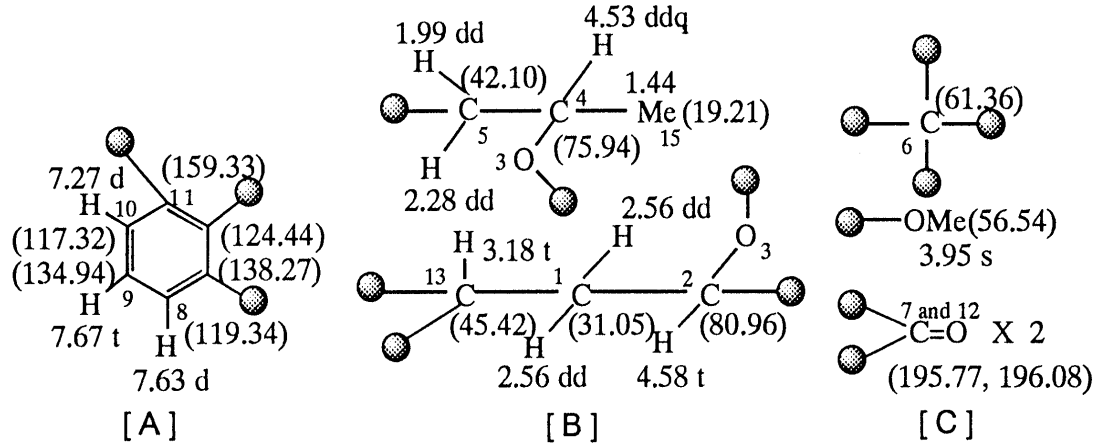

Fig. 1. Partial Structures Observed by ^1H and ^{13}C-NMR, ^1H-^1H COSY, and HMQC Spectra ($CDCl_3$, δ) of **9**. (^{13}C data in parentheses)

The connectivities of these partial structures were confirmed by the HMBC spectrum, leading to the partial structures [D and E] containing a novel four-membered ring and a tetrahydrofuran ring as shown by arrows in Fig. 2.

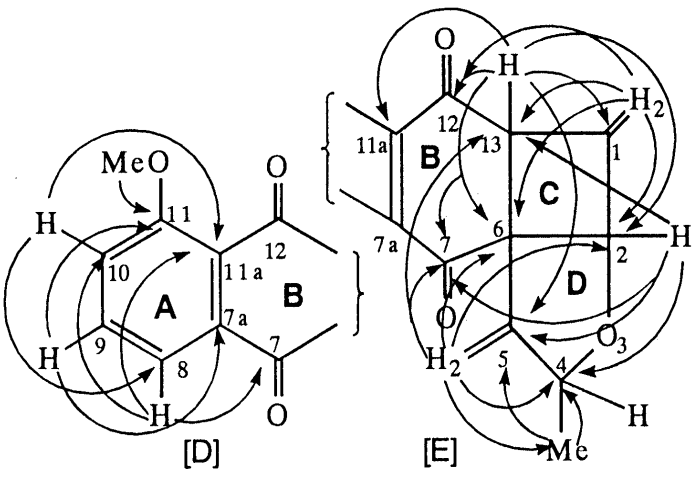

Fig. 2. HMBC Correlations [D and E] of **9**

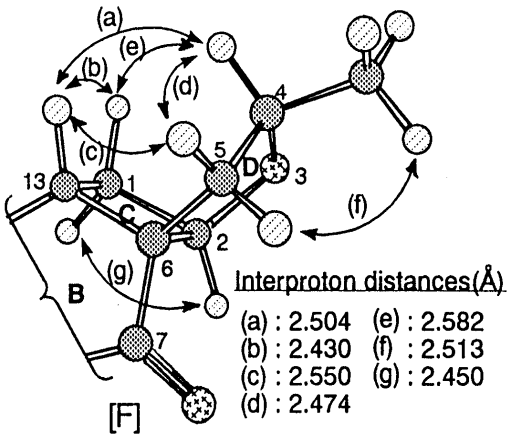

Fig. 3. NOE Correlations of B, C and D rings of **9**

The NOE correlations in the NOESY spectrum [$CDCl_3/C_6D_6$(4:1)] of elecanacin provided evidence for the B/C *cis* and C/D *cis* ring fusions, as depicted by arrows in structure [F] in Fig. 3. The NOEs were observed when the interproton distances ranged from 2.430—2.582 Å.[2] Thus the stereostructure of elecanacin was established as **9**. To our knowledge, **9** is the first example of a 1,4-naphtoquinone derivative with a four-membered ring and a tetrahydrofuran ring in the molecule.

The physical and spectral data[3] of Isoeleutherol (**10**) were similar to those of eleutherol (**1**),[1] and its planar structure was determined from extensive analysis of the 2D NMR spectra (^{1}H-^{1}H COSY, HMQC, and HMBC). The specific rotation ($CHCl_3$) of **1** is +83° , and that of **10** is -60.5°. Consequently, the stereostructure of isoeleutherol is assumed to be the same as that of the enantiomer of eleutherol (**1**).

The inhibitory activities of compounds **2**, **3**, **9**, and **10** against TOPO II and HIV were evaluated. For eleutherin (**2**) the IC_{100} TOPO II,[4a] EC_{50} KB cells,[4b] and EC_{50} -7d cells[4b] were 50, 14, and 5 μg/ml, respectively, and compounds **3**, **9**, and **10** showed no activity. Eleutherin (**2**) appears to be interesting as a "noncleavable complex" type of TOPO II inhibitor with stereospecific and selective activity. Isoeleutherin (**3**) and isoeleutherol (**10**) demonstrated inhibitory activity against HIV replication in H9 lymphocytes with IC_{50} and EC_{50} values[4c] of 8.55 and 100 μg/ml, and 1.41 and 7.44 μg/ml, respectively. Further studies on these and other constituents are now in progress and will be reported elsewhere.

REFERENCES AND NOTES

1) Chen Z., Huang H., Wang C., Li Y., Ding J., Sankawa U., Noguchi H., Iitaka Y., *Chem. Pharm. Bull.*, **34**, 2743-2746 (1986) and references cited therein.
2) The interproton distances (Å) were calculated using MM2 and MOPAC(PM3) parameters of CAChe (ver. 3.8).
3) Eleutherin (**2**): brown needles, mp 178～179 °C, $[\alpha]_D$ +220.7° ($CHCl_3$), $C_{16}H_{16}O_4$, IR($CHCl_3$,cm^{-1}): 1685, 1600, 1500, ^{1}H-NMR($CDCl_3$) δ: 1.36 (3H, d, *J*=6.5 Hz, 3-Me), 1.54 (3H, d, *J*=6.5 Hz, 1-Me), 2.20 (1H, dd, *J*=10.3, 18.1 Hz, 4-βH), 2.75 (1H, dd, *J*=2.4,18.1 Hz, 4-αH), 3.96 (1H, m, 3-H), 4.00 (3H, s, 9-OMe), 5.01 (1H, q, *J*=6.7 Hz, 1-H), 7.28 (1H, d, *J*=6.7 Hz, 8-H), 7.64 (1H t, *J*=6.7 Hz, 7-H), 7.74 (1H,d, *J*=6.7 Hz, 6-H).
Isoeleutherin (**3**): brown needles, mp 174～175 °C, $[\alpha]_D$ +14.6° ($CHCl_3$), $C_{16}H_{16}O_4$, IR($CHCl_3$, cm^{-1}): 1660, 1595, 1470, ^{1}H-NMR($CDCl_3$) δ: 1.34 (3H, d, *J*=6.1Hz, 3-Me), 1.53 (3H, d, *J*=6.7 Hz, 1-Me), 2.23 (1H, dd, *J*=11.0,19.0 Hz, 4-βH), 2.69 (1H,dd, *J*=3.5, 19.0 Hz, 4-αH),3.96 (1H, m, 3-H), 4.00 (3H, s, 9-OMe), 5.01 (1H, q, *J*=6.7Hz, 1-H), 7.27 (1H, d, *J*=6.7 Hz, 8-H), 7.64 (1H t, *J*=6.7Hz, 7-H), 7.74 (1H,d, J=6.7 Hz, 6-H).
Isoeleutherol (**10**): brown needles, mp 202～203 °C, $[\alpha]_D$ -60.5° ($CHCl_3$), $C_{14}H_{12}O_4$, IR ($CHCl_3$, cm^{-1}): 3400, 1765;^{1}H-NMR ($CDCl_3$) δ: 1.73 (3H, d, *J*=6.5 Hz, 1-Me),1.54 (3H, d,*J*=6.5 Hz, 1-Me), 4.11 (3H, s, 8-OMe), 5.70 (1H, q, *J*=6.5 Hz, 1-H), 6.93 (1H, d, J= 7.7 Hz, 7-H),7.39 (1H, t, *J*=7.7 Hz, 6-H), 7.54 (1H t, *J*=7.7 Hz, 5-H), 7.84 (1H,s, 4-H), 9.63 (1H, s, 9-OH);^{13}C-NMR ($CDCl_3$) δ: 19.18 (q, 11-C), 56.43 (q, 10-C), 76.64 (d, 1-C), 106.33 (d,7-C), 116.50 (d, 6-C),117.54 (s, 8a-C), 123.67 (d, 5-C), 125.92 (s, 3a-C), 126.61 (d, 4-C),127.94 (s, 9a-C), 137.24 (s, 4a-C), 149.20 (s, 9-C), 156.61 (s, 8-C), 170.55 (s, 3-C).
4) a) Tested versus calf thymus topoisomerase II-dependent DNA unknotting *in vitro*.
b) Tested for cell growth inhibition over continuous three-day exposure. The -7d cell line is resistant to VP-16 (a "cleavable-complex" type of topoisomerase II inhibitor) in part because it has reduced levels of enzyme.
c) TI (IC_{50} / EC_{50}) ≥ 5.0 considered to be significant.
Isoeleutherin (**3**): TI=6.07; isoeleutherol (**10**): TI=13.4

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