PERGAMON

Phytochemistry 50 (1999) 1385–1389

PHYTOCHEMISTRY

Epimers from the leaves of *Calophyllum inophyllum*☆

Muhammad Shaiq Ali[a,*], Shaukat Mahmud[b], Shaista Perveen[c], Viqar Uddin Ahmad[a], Ghazala Hafeez Rizwani[b]

[a]*H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi 75270, Pakistan*
[c]*Environmental Engineering Department, NED University of Engineering and Technology, Karachi 75270, Pakistan*

Received 28 May 1998

Abstract

The ethanolic extract of the fresh leaves of *Calophyllum inophyllum* afforded a pair of new epimers named as inophynone and isoinophynone. Their structures were elucidated with the aid of spectroscopic techniques. Some known constituents, cholesterol, friedelin, canophyllol and canophyllic acid, were also isolated from the same source.

Keywords: Calophyllum inophyllum Linn; Guttiferae; Leaves; Epimers; Structure elucidation; Spectroscopy; Bioactivity

1. Introduction

Calophyllum inophyllum Linn. is a common, moderate sized, evergreen, ornamental and medicinal garden-plant. In English, it is called 'Alexandrian Laurel'. The genus *Calophyllum* consists of about 130 species, distributed in tropical areas of the world, of which five species are cultivated in Pakistan, i.e. *C. inophyllum*, *C. apetalum*, *C. polyanthum*, *C. elatum* and *C. spectabil*.

The plant has great medicinal value and all parts (such as bark, leaves, seeds) are used as antiseptics, astringents, expectorants, diuretics and purgatives. The oil of the seeds and roots is beneficial in the treatment of wounds and scabies. The plant is also recommended in leprous nephritis, and is injected intramuscularly as an analgesic. Several inophyllums from *C. inophyllum* and calanolides from *C. lanigerum* have been isolated and found to be anti-HIV agents (Patil et al., 1993; Kashman et al., 1992).

The chemical literature reflects the existence of wide variety of natural products such as flavonoids (Ravelonjato, Kunesch, & Poisson, 1987), triterpenoids (Bandara, Dharmaratne, Sotheeswaran, & Balasubramaniam, 1986), xanthones (Dharmaratne, Sotheeswaran, Balasubramaniam, & Reisch, 1986), coumarines (Dharmaratne, Sotheeswaran, Balasubramaniam, & Waight, 1985), steroids (Banergi & Nigam, 1977) and other bioactive compounds. Although some of the *Calophyllum* species have been examined phytochemically but no intensive research work has been reported in the literature on *C. inophyllum*.

In view of the medicinal importance and availability in Pakistan, we have investigated *C. inophyllum*, and isolated a pair of new epimers (**1** and **2**) and some known constituents, all of which were characterized with the help of spectroscopic techniques including 2D-NMR.

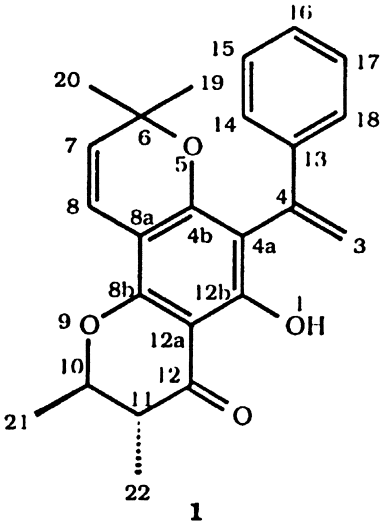
2. Results and discussion

The ethanolic extract of the fresh leaves of *C. inophyllum* was partitioned between chloroform and water. From the chloroform-soluble part two new compounds (**1** and **2**) were isolated.

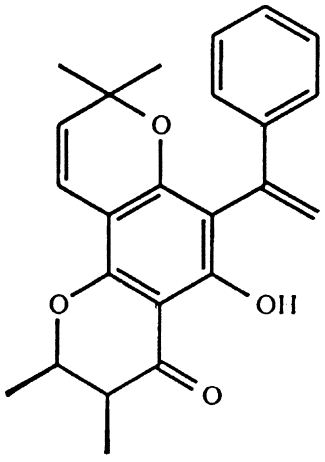
☆ Dedicated to the memory of Hakim Mohammed Said.

* Corresponding author.

0031-9422/99 $ - see front matter
PII: S0031-9422(98)00480-4



1



2

The IR spectrum of **1** displayed a strong absorption band at 1630 cm^{-1} indicative of the presence of an α,β-unsaturated ketone in the molecule. The EI mass spectrum showed the $[M]^+$ peak at *m/z* 376. The same spectrum also exhibited the base peak at *m/z* 361 together with a peak at *m/z* 103 due to the losses of a methyl and C_8H_7 (moiety substituted at C4a) from the $[M]^+$ peak, respectively. The molecular formula of **1** was established by HRMS as $C_{24}H_{24}O_4$ (*m/z* 376.16509 requires 376.16744). The molecular formula showed 13 degrees of unsaturation, one of which was due to a ketone function.

The ^1H NMR spectrum of **1** showed the presence of four methyl signals, of which two appeared at δ 1.53 and 1.22 as doublets having coupling constants of 6.54 and 7.31 Hz. These were assigned to Me-21 and Me-22, respectively. The other gem-dimethyl singlets appeared at δ 1.11 and 1.16 and were attributable to Me-19 and M-20, respectively. The two methine signals at δ 5.43 ($J = 9.99$ Hz) and 6.55 ($J = 9.99$ Hz) appeared as sharp doublets and were assigned to H-7 and H-8, respectively. The coupling constants of these signals established that they were vicinal to each other. The *dq* of signals at δ 4.28 for H-11 was due to Me-22 and also the neighbouring CH (H-10). The orientation of Me-22 in compound **1** was assigned as α by comparison with the reported data of related compounds (Patil et al., 1993; Kashman et al., 1992). Another CH signal which appeared as a *dq* at δ 4.60 was assigned to H-10. This multiplicity (*dq*) being due to Me-21 and the adjacent CH (H-11). A 3H signal at δ 7.25 appeared as a multiplet and was due to the aromatic protons (H-14, H-16 and C-18). A 2H signal at δ 7.34 also appeared as a multiplet and was assigned to H-15 and H-17. The most downfield and confusing signal resonated at δ 12.57 in the form of a sharp singlet. This was due to the hydroxyl proton. It was thought that this downfield proton may be due to an acidic function. However, as no reaction was observed when it was treated with diazomethane **1** does not contain a carboxyl function and thus the proton at δ 12.57 was due to a hydroxyl proton. This proton is involved in chelation and this is the reason that a hydroxyl function did not appear in the IR spectrum (Fig. 1).

The ^1H NMR spectrum also exhibited a pair of doublets at δ 5.28 and 5.85 ($J = 1.47$ Hz). These were assigned to the olefinic-methylene moiety. As the molecule does not contain any other methylene group this was further confirmed through a DEPT experiment (δ 117.7). In this way, the second degree of unsaturation was accounted for.

The DEPT experiment also showed the presence of nine methine signals, most of them in the downfield region and indicative of the presence of an aromatic

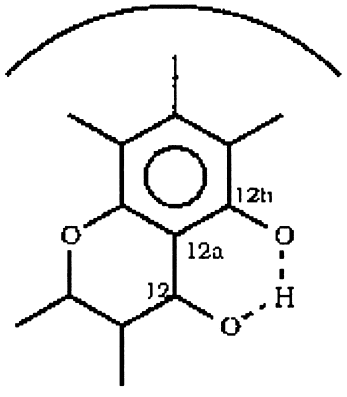


Fig. 1.

Table 1
^{13}C NMR data for compounds **1** and **2** (75.4 MHz, $CDCl_3$)

C	1	2
3	117.7	117.7
4	139.6	139.6
4a	111.0	111.0
4b	159.4	159.4
6	77.8	77.9
7	126.4	126.3
8	115.6	115.7
8a	101.4	101.0
8b	161.1	161.5
10	79.0	76.3
11	45.7	44.4
12	198.7	200.0
12a	101.7	101.7
12b	155.8	155.8
13	141.6	141.6
14	126.2	126.2
15	128.0	128.0
16	127.1	127.1
17	128.7	128.7
18	126.2	126.2
19	27.6	27.8
20	28.0	28.0
21	19.6	16.5
22	10.1	9.2

moiety/moieties in the molecule. The same experiment further resolved the presence of four methyls and 10 quaternary carbon atoms. The four methyls gave rise to signals at δ_C 10.1, 19.6, 27.6 and 28.0 and δ_H 1.22 (*d*, $J = 7.31$ Hz, H-22), 1.53 (*d*, $J = 6.54$ Hz, H-21), 1.11 (*s*, H-19) and 1.16 (*s*, H-20), respectively. The only carbonyl carbon appeared at δ 198.7 and was due to the α,β-unsaturated ketone. Another downfield quaternary carbon resonated at δ 139.6 and was assigned to the carbon associated with the olefinic-methylene carbon. A signal at δ 159.4 was due to the aromatic ring quaternary carbon directly attached to oxygen and assigned for C-4b. Another quaternary carbon directly attached to oxygen appeared at δ 161.1 and was assigned to C-8b. The signals at δ 155.8 were due to the carbon-containing hydroxyl function. The two upfield methine signals at δ 79.0 and 45.7 in the DEPT spectrum were due to C-10 and C-11, respectively. The signals due to the endo-cyclic double bond resonated at δ 126.4 (C-7) and 115.6 (C-8). The remaining quaternary and aromatic methine signals (Table 1) were compared with the reported data (Patil et al., 1993; Kashman et al., 1992). The various protons and their associated carbons were located through a hetero-COSY experiment. Similarly, the ^1H–^1H connectivities were also established through decoupling and COSY-45 techniques.

On the basis of the spectral details and comparison with the reported data (Patil et al., 1993; Kashman et al., 1992), the structure of the new compound was assigned as **1** and named as 'inophynone'. Compound **1** did not show any significant response when tested against various gram −ve and +ve bacteria and various animal, plant and human pathogens. This compound has not been reported so far from any natural source. The olefinic moiety between C-3 and C-4 can be generated as a result of decarboxylation (Fig. 2).

Compound **2** was almost identical with compound **1** in all respects except at positions C-10 and C-11. In the ^1H NMR spectrum of compound **2**, Me-22 appeared at δ 1.22 as a doublet ($J = 6.69$ Hz) but its associated carbon resonated at δ 9.2 instead of δ 10.1 as observed in compound **1**. This shows that Me-22 must have reverse stereochemistry to that of Me-22 of **1** and should be 'β'. The big difference in the chemical shifts of positions C-10, -11 and -12 can be seen in Table 2.

In case of isoinophynone (**2**) the signal of H-11 resonated at δ 2.59 (dq, $J = 11.23$, 6.69 Hz) instead of δ 4.28 (*dq*, $J = 7.31$, 3.22 Hz) as observed in inophynone (**1**). Similarly, the signals of H-10 appeared at δ 4.23 in **2** instead of δ 4.60 as in **1**. The magnitudes of the coupling constants in these signals clearly established (Table 2) that **1** and **2** have an epimeric relationship

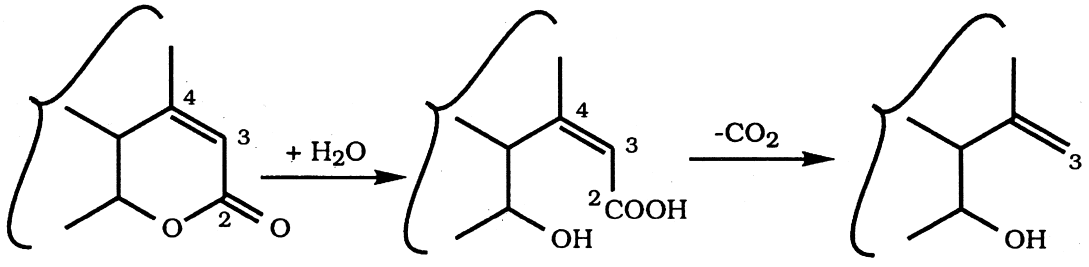
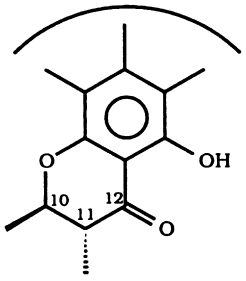
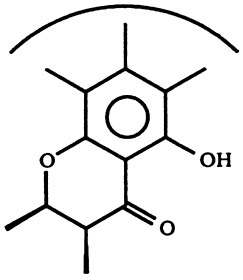


Fig. 2.

Table 2
Chemical shift data for H-10 and -11 and C-10 to C-12

Atom	1	2
H-10	4.60 (1H, *dq*, *J* = 6.54, 3.21 Hz)	4.23 (1H, *dq*, *J* = 11.23, 6.24 Hz)
H-11	4.28 (1H, *dq*, *J* = 7.31, 3.22 Hz)	2.59 (1H, *dq* *J* = 11.23, 6.69 Hz)
C-10	79.0	76.3
C-11	45.7	44.4
C-12	198.7	200.0





with respect to position C-11. As the stereochemistry of Me-22 is assigned as α in **1** based on the literature (Patil et al., 1993), Me-22 must be β oriented in **2**.

Due to the change in orientation of Me-22 in **2** the effect is transferred towards C-10 and C-21 which showed their resonances at δ 76.3 and 16.5, respectively, instead of at δ 79.0 and 19.6 as in the carbon spectrum of **1**. On the basis of the change in orientation of Me-22, compound **2** is named as *Isoinophynone*.

This new pair of epimers (**1** and **2**) have not been isolated from any natural source.

3. Experimental

NMR: 400 and 300 MHz (^{1}H), and 100 and 75 MHz (^{13}C).

3.1. Collection and identification

Fresh leaves of *Calophyllum inophyllum* Linn. (5 kg) were collected from Karachi region and identified by Dr. Suryyia Khatoon, Assistant Professor, Department of Botany, University of Karachi, Karachi, where the voucher specimen (No. 9170) of the plant is deposited in the herbarium.

3.2. Extraction and isolation

Fresh leaves of *C. inophyllum* were chopped into small pieces and soaked in EtOH for 15 days. The EtOH was removed by evaporation under reduced pressure. The gummy ethanolic extract (95 g) thus obtained, was diluted with H_2O and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ was removed under reduced pressure and the residue (41.2 g) was subjected to VLC. Various fractions were taken using mixtures of hexane, $CHCl_3$ and MeOH. The fraction eluted with MeOH–$CHCl_3$ (1:4) from VLC was subjected to CC. A solid material was obtained with $CHCl_3$–hexane which was washed with hexane to give **1** as fine crystals (75 mg): MP 115°; IR ($CHCl_3$) ν_{max} cm^{-1}: 1630 (α,β-unsaturated ketone), 1442 and 1125; EI–MS: *m*/*z* 376 $[M]^+$, 361 $[M-Me]^+$ (100%), 305, 377, 277, 202, 188, 165, 155, 103 and 77; HR-MS: *m*/*z* 376.16509 ($C_{24}H_{24}O_4$ requires 376.16744); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.57 (1H, *s*, OH-1) 5.28 (1H, *d*, *J* = 1.47 Hz, H-3), 5.85 (1H, *d*, *J* = 1.47 Hz, H-3′), 5.43 (1H, *d*, *J* = 9.99 Hz, H-7), 6.55 (1H, *d*, *J* = 9.99 Hz, H-8), 4.60 (1H, *dq*, *J* = 6.54, 3.21 Hz, H-10), 4.28 (1H, *dq*, *J* = 7.31, 3.22 Hz, H-11), 7.25 (3H, *m*, H-14, 16, 18), 7.34 (2H, *m*, H-15, 17), 1.11 (3H, *s*, H-19), 1.16 (3H, *s*, H-20), 1.53 (3H, *d*, *J* = 6.54 Hz, H-21), 1.22 (3H, *d*, *J* = 7.31 Hz, H-22); ^{13}C NMR: Table 1.

Elution with the same solvent system gave another crystalline compound (**2**) (13.13 mg): MP: 185°; IR ($CHCl_3$) ν_{max} cm^{-1}: 1638 (α,β-unsaturated ketone), 1445 and 1135; EI–MS: *m*/*z* 376 $[M]^+$, 361

$[M-Me]^+$ (100), 305, 377, 227, 202, 188, 165, 149, 103 and 77; HRMS: *m/z* 376.16623 ($C_{24}H_{24}O_4$ requires 376.16744); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.51 (1H, *s*, OH-1), 5.28 (1H, *d*, *J* = 1.47 Hz, H-3), 5.85 (1H, *d*, *J* = 1.47 Hz, H-3), 5.48 (1H, *d*, *J* = 9.99 Hz, H-7), 6.53 (1 H, *d*, *J* = 9.99 Hz, H-8), 4.23 (1H, *dq*, *J* = 11.23, 6.24 Hz, H-10), 2.59 (1H, *dq*, *J* = 11.23, 6.69 Hz, H-11), 7.23 (3H, *m*, H-14, 16, 18), 7.37 (2H, *m*, H-15, 17), 1.14 (3H, *s*, H-19), 1.18 (3H, *s*, H-20), 1.42 (3H, *d*, *J* = 6.27 Hz, H-21) and 1.22 (3H, *d*, *J* = 6.69 Hz, H-22); ^{13}C NMR: Table 1.

3.3. Cholesterol (Kikuchi, Suzuki, Suzuki, & Kurosawa, 1985)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.51 (*m*, $W_{1/2}$ = 15.20 Hz, H-3), 5.35 (distorted *t*, H-6), 0.67 (*s*, H-18), 1.00 (*s*, H-19), 0.91 (3H, *d*, *J* = 6.56 Hz, H-21) and 0.86 (*d*, *J* = 6.64 Hz, H-26 and H-27).

3.4. Friedelin (Klass & Tinto, 1992; Umehara et al., 1988)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.85 (3H, *d*, *J* = 6.5 Hz, H-23), 0.71 (3H, *s*, H-24), 0.86 (3H, *s*, H-25), 1.00 (3H, *s*, H-26), 1.05 (3H, *s*, H-27), 1.17 (3H, *s*, H-28), 1.00 (3H, *s*, H-29) and 0.95 (3H, *s*, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.27 (C-1), 41.54 (C-2), 213.17 (C-3), 58.19 (C-4), 42.15 (C-5), 41.27 (C-6), 18.30 (C-7), 53.08 (C-8), 37.55 (C-9), 59.45 (C-10), 35.61 (C-11), 30.50 (C-12), 39.68 (C-13), 38.28 (C-14), 32.76 (C-15), 36.00 (C-16), 30.06 (C-17), 42.77 (C-18), 35.32 (C-19) 28.19 (C-20), 32.40 (C-21), 39.31 (C-22), 6.78 (C-23), 14.68 (C-24), 17.96 (C-25), 20.26 (C-26), 18.64 (C-27), 32.14 (C-28), 31.82 (C-29) and 35.01 (C-30).

3.5. Canophyllol (Ahmad & Rahman, 1994; Patra & Chaudhuri, 1987)

EI–MS: *m/z* $[M]^+$ 442 (2%) and 137 (100); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.62(2H, *s*, H-28) 2.50, 2.20 (3H, *m*, H-4, 2α, 2β), 1.14[1], 0.99, 0.98 and 0.91 (each 3H, H-30, 29, 26, 27), 0.87 (3H, *d*, *J* = 6.0 Hz, H-23) and 0.87 and 0.72 (each 3H, *s*, H-25, 24); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.27 (C-1), 41.48 (C-2), 212.73 (C-3), 58.26 (C-4), 42.08 (C-5), 41.32 (C-6), 18.28 (C-7), 52.55 (C-8), 37.52 (C-9), 59.57 (C10), 35.50 (C-11), 30.13 (C-12), 39.42 (C-13), 38.20 (C-14), 31.39 (C-15), 29.16 (C16), 35.22 (C-17), 39.50 (C-18), 34.57 (C-19), 28.13 (C-20), 31.49 (C-21), 33.44 (C-22), 6.79 (C-2.3), 14.68 (C-24), 18.07 (C-25), 19.08 (C-26), 19.17 (C-27), 68.04 (C-28), 34.27 (C-29) and 32.84 (C-30).

3.6. Canophyllic acid (Patra & Chaudhuri, 1987; Govindachari, Viswanathan, Pai, Rao, & Srinivasan, 1967)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (3H, *d*, *J* = 6.52 Hz, H-23), 0.70 (3H, *s*, H-24), 0.85 (3H, *s*, H-25), 0.80 (3H, *s*, H-26), 1.02 (each 3H, *s*, H-27, 30) and 0.92 (3H, *s*, H-29); ^{13}C NMR ($CDCl_3$, 75.43 MHz): δ 22.29 (C-1), 41.52 (C-2), 212.68 (C-3), 58.34 (C-4), 42.09 (C-5), 41.26 (C-6), 18.18 (C-7), 53.15 (C-8), 37.82 (C-9), 59.48 (C-10), 35.98 (C-11), 31.11 (C-12), 39.02 (C-13, 14), 32.80 (C-15), 34.95 (C-16), 44.84 (C-17), 37.95 (C-18), 35.60 (C-19), 28.47 (C-20), 32.62 (C-21), 29.55 (C-22), 6.77 (C-23), 14.69 (C-24), 17.53 (C-25), 20.62 (C-26), 18.51 (C-27), 183–86 (C-28), 29.81 (C-29) and 34.52 (C-30).

References

Ahmad, V. U., & Rahman, A. U. (1994). *Hand Book of Natural Product Data, 2,* 586.

Bandara, B. M. R., Dharmaratne, H. R. W., Sotheeswaran, S., & Balasubramaniam, S. (1986). *Phytochemistry, 25,* 425.

Banergi, R., & Nigam, S. K. (1977). *Q. J. Crude Drug Res., 15,* 133.

Dharmaratne, H. R. W., Sotheeswaran, S., Balasubramaniam, S., & Waight, E. S. (1985). *Phytochemistry, 24,* 1553.

Dharmaratne, H. R. W., Sotheeswaran, S., Balasubramaniam, S., & Reisch, J. (1986). *Phytochemistry, 25,* 1957.

Govindachari, T. R., Viswanathan, N., Pai, B. R., Rao, U. R., & Srinivasan, M. (1967). *Tetrahedron, 23,* 1901.

Kashman, Y., Gustafson, K. R., Fuller, R. W., Cardellina, J. H., Mahon, J. B., Currens, M. J., Buckheit, R. W., Hughes, S. H., Cragg, G. M., & Boyd, M. R. (1992). *J. Med. Chem., 35,* 2735.

Kikuchi, H., Suzuki, T., Suzuki, M., & Kurosawa, E. (1985). *Bull. Chem. Soc., 58,* 2437.

Klass, J., & Tinto, W. F. (1992). *J. Nat. Prod., 55,* 1626.

Patil, A. D., Fryer, A. J., Eggleston, D. S., Haltiwanger, R. C., Bean, M. F., Taylor, P. B., Caranfa, M. J., Breen, A. L., Bartus, H. R., Johnson, R. K., Hertzberg, R. P., & Westley, J. W. (1993). *J. Med. Chem., 36,* 1431.

Patra, A., & Chaudhuri, S. K. (1987). *Magn. Reson. Chem., 25,* 95.

Ravelonjato, B., Kunesch, N., & Poisson, E. J. (1987). *Phytochemistry, 26,* 2973.

Umehara, K., Hattori, I., Miyase, T., Ueno, A., Hara, S., & Kageyama, C. (1988). *Chem. Pharm. Bull., 36,* 5004.

[1] Assignment may be interchanged.