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TIRUCALLANE TRITERPENES FROM THE STEM BARK OF *AGLAIA LEUCOPHYLLA*

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Key Word Index—*Aglaia leucophylla*; Meliaceae; stem bark; tirucallane triterpenes.

Abstract—A new tirucallane triterpene, (−)-leucophyllone, was isolated from the stem bark of *Aglaia leucophylla* and its structure was elucidated from spectral data as 23(*Z*)-25-methoxy-tirucall-7,23-diene-3-one. In addition, (−)-caryophyllene oxide, (−)-niloticin, (−)-bourjotinolone and (−)-piscidinol were isolated from the same plant.

INTRODUCTION

We recently showed that (+)-ocotillone was responsible for the cytotoxic activity of an ethanolic extract of *Aglaia leucophylla* King against KB cells [1]. It then became of interest to evaluate further the bioactivity of this compound in different *in vitro* test procedures. However, to the small amount of (+)-ocotillone isolated in our previous work, it was necessary to collect a new specimen of the Malaysian *Aglaia* in order to extract further amounts of the bioactive compound. In this paper, we report on the isolation of the new compound **1** and the known compounds **2**–**5** from dried stem bark of *A. leucophylla*.

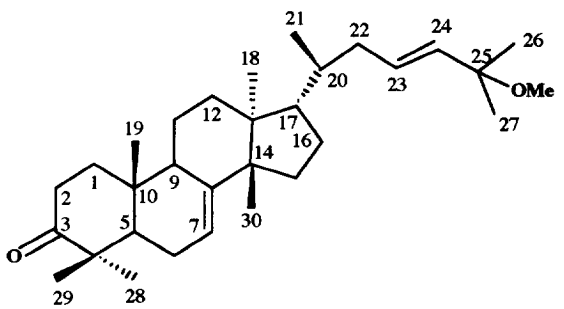
RESULTS AND DISCUSSION

The dried powdered stem bark of *A. leucophylla* was extracted and chromatographed as previously described [1]. Five compounds were isolated and identified by means of spectroscopic methods as new (−)-caryophyllene oxide (**2**), (−)-niloticin (**3**), (−)-bourjotinolone (**4**), (−)-piscidinol (**5**) and the new compound (−)-leucophyllone (**1**).

Compound **1** was assigned the molecular formula $C_{31}H_{50}O_2$ (HR EI-MS: calcd: 454.83811; found: 454.3821). An IR absorption band at 1709 cm^{-1} suggested it to be a keto-triterpenoid [2]. The mass spectrum of **1** also exhibited fragment ions characteristic of a tirucallane skeleton [3] at *m/z* 439 $[M - Me]^+$, 271 $[C_{19}H_{27}O]^+$ and 138 $[C_9H_{14}O]^+$, the latter being attributable to a retro Diels–Alder cleavage of ring B (Scheme 1). The ^1H NMR spectrum of **1** showed methyl resonances at δ0.82 (3H, *s*, Me-18), 0.88 (3H, *d*, J = 6.0 Hz, Me-21), 1.01 (6H, *s*, Me-19 and Me-30), 1.12 (3H, *s*, Me-28) and 1.26 (6H, *s*, Me-26 and Me-27).

Signals for olefinic protons were observed at δ5.30 (1H, *m*, H-7) [4], 5.40 (1H, *d*, J = 16.0 Hz, H-24) and 5.60 (1H, *ddd*, J = 16.0, 8.0 and 5.6 Hz, H-23) whereas the signal of a methoxyl group was seen at δ3.16 (3H, *s*, OMe-25). The large coupling constant (16.0 Hz) between H-23 and H-24 was indicative of a *Z*-geometry of the side-chain double bond.

Based on the ^{13}C and DEPT NMR spectra, it was also shown that this triterpenoid possessed eight methyl groups, eight methylenes, seven methines and one carbonyl group. The signals for C-8 and C-7 appeared at δ145.8 and 117.8, respectively [5]. These chemical shifts are characteristic of the Δ^7-euphane and the Δ^7-tirucallane series [6]. The strongly negative optical rotation of **1** indicated that it belonged to the tirucallane series [7]



1

Scheme 1

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Table 1. ^{13}C NMR spectral data and atom type from DEPT for **1** ($CDCl_3$, 67.5 MHz)

Carbon no.	Atom type	δ (ppm)	Carbon no.	Atom type	δ (ppm)
1	CH_2	38.5	17	CH	52.5
2	CH_2	34.9	18	CH_3	21.9
3	C	216.9	19	CH_3	12.7
4	C	47.8	20	CH	36.3
5	CH	52.3	21	CH_3	18.4
6	CH_2	24.3	22	CH_2	39.1
7	CH	117.8	23	CH	128.6
8	C	145.8	24	CH	136.6
9	CH	48.4	25	C	74.8
10	C	34.9	26	CH_3	25.7
11	CH_2	18.2	27	CH_3	26.2
12	CH_2	34.0	28	CH_3	24.5
13	C	43.4	29	CH_3	21.6
14	C	51.1	30	CH_3	27.4
15	CH_2	33.5	31	OCH_3	50.2
16	CH_2	28.0			

and assignment of each carbon resonance of the molecule was completed through ^1H–^{13}C COSY and HMBC experiments (Table 1). In this way, **1** was identified as 25-methoxy-tirucall-7-23 (*trans*)-diene-3-one, which we have named (−)-leucophyllone.

Thus, this plant only contains tirucallane derivatives, whereas secotirucallane and dammarane derivatives were isolated from our first specimen of *A. leucophylla*. This difference between the two specimens collected in the Malaysian peninsula prompted us to reinvestigate the botanical identity of each plant. Careful examination of the fruit from samples of each specimen recollected during fructification showed that the former specimen actually belonged to a genus closely related to *Aglaia* in the Meliaceae family—*Dysoxylum*—and this plant was finally identified as *Dysoxylum cauliflorum* Hiern. This name should thus replace the erroneous *Aglaia leucophylla* used in our earlier paper [1]. As far as biological properties are concerned, it should also be noted that the ethanolic extract of *A. leucophylla* does not exhibit cytotoxic activity, unlike *D. cauliflorum*, nor anti-HIV, antifungal or antibacterial activity.

EXPERIMENTAL

Extraction and fractionation. The plant material was collected by one of us (G.P.) in Lumut, Perak, Malaysia, in November 1992. A herbarium specimen is deposited at the Laboratoire de Phanérogamie, MNHN, Paris, and at the University of Malaya, Kuala-Lumpur, under the reference KL4175.

The stem bark (3.0 kg) was air-dried, powdered and allowed to stand in MeOH at room temp. The extract was filtered and evapd to dryness to yield an oil (222.6 g). This extract was then partitioned between aq. MeOH and hexane. The hexane extract (19.3 g) on MPLC on silica gel (40–63 µm mesh) gave caryophyllene oxide (2.16 g; fr 1–13, *n*-hexane–EtOAc 47:3), niloticin (7.85 g; frs 20–49, 3:1), leucophyllone (497 mg; frs 50–55, 4:1), bourjotinolone (325 mg; frs 56–61, 3:2) and piscidinol (193 mg; frs 62–67, $CHCl_3$–MeOH, 99:1).

Compound (**1**) (*25-methoxy-tirucall-7-23* (trans)-*diene-3-one*. $[\alpha]_D - 95$ ($CHCl_3$; c1); HR-MS 70 ev, *m/z*: 454.3821 $[M]^+$ (calc. for $C_{31}H_{50}O_2$, 454.3811); IR ν^{KBr} cm^{-1}: 1709 (CO, carbonyl), 1076 (CO, ether); EI-MS 70 ev, *m/z* (rel. int.): 454 (10) $[M]^+$, 439 (10) $[M - Me]^+$, 423 (2) $[M - OMe]^+$, 407 (100), 325 (25) $[C_{23}H_{33}O]^+$, 271 (9) $[C_{19}H_{27}O]^+$, 175 (16), 138 (2), 123 (22), 113 (8), 81 (37) $[C_6H_9]^+$, 73 (23) $[C_4H_5O]^+$, ^1H NMR (270 MHz, $CDCl_3$, TMS = 0): δ 5.50 (*ddd*, *J* = 15.7, 8, 5.6 Hz, H-23), 5.40 (*d*, *J* = 16 Hz, H-24), 5.30 (*m*, H-7), 3.16 (*s*, OMe), 2.76 (*ddd*, *J* = 14, 5.4 Hz; H-2ax), 1.26 (*s*, Me-26 and Me-27), 1.12 (*s*, Me-28), 1.05 (*s*, Me-29), 1.01 (*s*, Me-19 and Me-30), 0.88 (*d*, *J* = 6 Hz, Me-21), 0.82 (*s*, Me-18); ^{13}C NMR: Table 1.

Caryophyllene oxide (**2**). EI-MS 70 ev, *m/z*: 220 $[M]^+$ $C_{15}H_{24}O$; ^1H NMR (270 MHz, $CDCl_3$, TMS = 0): δ 4.96–4.85 (2*br s*, 2H, C=CH_2), 2.88 (*dd*, *J* = 10.6, 4 Hz, 1H, C–O–CH), 1.2 (*s*, 3H, C–O–C-CH_3) 1.00 and 0.98 (2*s*, 6H, $C(CH_3)_2$.

Niloticin (**3**). $[\alpha]_D - 60$ ($CHCl_3$; *c*1); IR ν^{KBr} cm^{-1}: 3395 (OH), 1707 (CO); EI-MS 70 ev, *m/z* (rel. int.): 456 (12) $[M]^+$, 441 (5) $[M - Me]^+$, 369 (82) $[C_{25}H_{37}O_2]^+$, 325 (56) $[M\text{-}C_6H_{12}O_2\text{-}Me]^+$, 143 (15), 138 (6), 71 (24).

Bourjotinolone (**4**). $[\alpha]_D - 60$ ($CHCl_3$; *c*1); IR ν^{KBr} cm^{-1}: 3404 (OH), 1707 (CO); EI-MS 70 ev, *m/z* (rel. int.): 472 (19) $[M]^+$, 457 (9) $[M - Me]^+$, 439 (22) $[M\text{-}Me\text{-}H_2O]^+$, 413 (9) $[M\text{-}C_3H_7O]^+$, 396 (39) $[M\text{-}C_3H_7O\text{-}OH]^+$, 381 (100) $[M\text{-}C_3H_7O\text{-}Me\text{-}OH]^+$, 271 (5) $[C_{19}H_{27}O]^+$, 159 (11) $[C_8H_{15}O_3]^+$, 59 (44) $[C_3H_7O]^+$.

Piscidinol (**5**). $[\alpha]_D - 76$ ($CHCl_3$; *c*1); IR ν^{KBr} cm^{-1}: 3450 (OH), 1710 (CO); EI-MS 70 ev, *m/z* (rel. int.): 474 (10) $[M]^+$, 459 (3) $[M - Me]^+$, 441 (12) $[M\text{-}Me\text{-}H_2O]^+$, 423 (7) $[M\text{-}Me\text{-}2H_2O]^+$, 369 (100), 325 (53) $[C_{23}H_{33}O]^+$, 271 (12.5) $[C_{19}H_{27}O_3]^+$, 161 (23) $[C_8H_{117}O_3]^+$, 143 (10) $[C_8H_{17}O_3 - H_2O]^+$, 59 (40) $[C_3H_7O]^+$ 41 (68) $[C_3H_7O - H_2O]^+$.

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