A New Type of Stilbene-Related Secondary Metabolite, Idenburgene, from Cryptocarya idenburgensis

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A new type of natural product, idenburgene (1), was isolated from Cryptocarya idenburgensis, and its unique structure was elucidated. Four known compounds, 3-hydroxy-5-methoxystilbene (2), 2',6'-dihydroxy-4'-methoxystilbene (3), stigmaster-4-ene-3-one, and β-sitosterol were also isolated and identified.

Key words Cryptocarya idenburgensis; Lauraceae; idenburgene; stilbene; 3-hydroxy-5-methoxystilbene; 2',6'-dihydroxy-4'-methoxystilbene

The genus Cryptocarya belonging to the Lauraceae family is known as a rich source of secondary metabolites such as flavonoids, pyrones, lignans, terpenoids and alkaloids. Some compounds obtained from this genus show antitumor activity: cryptocaryone, goniothalamin, kamahar lactone, and kurzilaactone which were respectively isolated from C. bourdilloni 1) and C. laevigata, 2) C. calounera, 3) C. kamahar, 4) and C. kurzii. 5) During our chemical study of this genus, 2) 6) Cryptocarya idenburgensis ALLEN, which is a big tree growing in the forests of Irian Jaya Island, Indonesia, at altitude ca. 1400 m, was examined because the species has not been previously investigated chemically. According to Kosterman, 9) the synonym for this species is Cryptocarya gonioaclada KAN. & HAT.

A new compound, idenburgene (1), was obtained as a yellow viscous solid from the CHC13 extract of the stem bark of Cryptocarya idenburgensis. The HR-FABMS spectrum displayed an M+ ion at m/z 442.2126 corresponding to a molecular formula C22H28O4. The 1H-NMR spectrum exhibited the presence of 29 carbons including a carbonyl group (δ 174.2). The partial structures in 1 were analyzed from the spectroscopic data. The first partial structure, A, is a stilbene moiety. In the 1H-NMR spectrum, the signals appeared at δH 7.03 (d, J=16.1 Hz, H-7) and 7.05 (d, J=16.1 Hz, H-8) and were assigned to a pair of trans-olefinic protons. The signals at δH 6.62 (H-2, d, J=16.7 Hz) and 6.66 (H-6, d, J=1.6 Hz) were apparently two meta coupled aromatic protons. The 1H-NMR also showed the presence of a methoxy group and a hydroxyl group at the benzene ring, which resonated at δH 3.88 (s) and 5.20 (s), respectively. The signals at δH 7.26 (H-4', m), 7.35 (H-3' and H-5'), m and 7.50 (H-2' and H-6', br d, J=7.6 Hz) indicated the presence of a monosubstituent benzene group. The HMBC spectrum showed a correlation between an olefinic proton at H-8 and aromatic carbons at δC 126.5 (C-2' and C-6') and also between H-7 and aromatic carbons at δC 102.0 (C-6') and 107.9 (C-2). The 1H- and 13C-NMR data of this stilbene moiety in 1 were very similar to those of 2, which was isolated together with 1, except for the data at the C-4 position. From the above observations, the presence of a 3-hydroxy-5-methoxystilbene moiety in 1 was suggested.

Additionally, the presence of methylene protons was observed at δH 1.59 (H-3', m) and 1.67 (H-3', m) that were coupled to two other methylenes at δH 1.90 (H-4', m) and 2.35 (H-2', m). The HMBC spectrum showed correlations between a carbonyl carbon at δC 174.2 (C-1') and the protons at H-2', H-3', and a methoxy group (δ 3.65), which confirmed the presence of an n-butyrionic acid methyl ester moiety in 1 as a second partial structure, B.

The existence of another pair of trans-olefinic protons was identified from the signals at δH 6.52 (H-7', d, J=1.5, 16.1 Hz) and 6.63 (H-6', br d, J=16.1 Hz), which were coupled with each other. Furthermore, the presence of the second monosubstituent benzene group was revealed from 1H-NMR, which displayed signals at δH 7.19 (H-11', br d, J=7.3, 7.3 Hz), 7.25 (H-10' and H-12', m) and 7.36 (H-9' and H-13', m). The cross peaks in the HMBC spectrum exhibited a correlation between the proton at H-7' and carbons at δC 126.2 (C-9' and C-13') and H-6' with C-8' (δC 137.3) confirming that the trans-olefin group was connected with the benzene ring to form a styrene structural moiety. The H-1'-H-11' correlation between the olefinic proton (H-6') and the methine proton at δH 4.21 (H-5') in the COSY spectrum as well as the connectivity between H-7' and C-5' in the HMBC spectrum enabled us to construct the third partial structure C in 1 as a phenylpropanoid unit (Ph-CH=CH-CH=)

As we clarified the basic structures of three units (A, B and C) in 1 (Fig. 1), we next constructed the full structure using the HMBC spectrum (Fig. 2), as follows. Both the protons at H-3' and H-4' correlated to C-5', which indicated the attachment of the terminal carbon of an n-butyrionic acid moiety to C-5' in a phenylpropanoid moiety. A correlation appearing between protons at H-5' and carbons at δC 117.2 (C-4'), 158.6 (C-5) and 155.1 (C-3) confirmed that the C-4 position of the stilbene moiety was also attached to C-5'. Further, cross peaks between H-4' and C-4', between H-4' and C-6' (δC 132.3), and also between H-6' and C-4', confirmed the connecting position described above. The proposed structure 1 was strongly supported by the EI-MS data, which showed the existence of a molecular ion peak at m/z 442 (28%) and the loss of an aliphatic ester molecule (C9H16O2), which appeared at m/z 341 as the base peak. The CD spectrum of 1 revealed a cotton effect due to a chiral center, but its absolute configuration is unknown at this stage.

We are interested in understanding the biosynthetic construction of this unique molecule (1). The structure of 1 can be dissected at the bond between C-4 and C-5' into two
parts, a stilbene unit and the remaining half. It is quite obvious that the stilbene part is biosynthesized through condensation of a cinnamoyl-CoA with three units of malonate followed by ring closure. Intensive enzymatic studies have been done on so-called CHS-super families, the enzymes that catalyze formation of chalcones, stilbenes and other polyketides in higher plants. Obviously similar enzymes are in operation in this plant to form the stilbene part of 1. More interesting is the biosynthetic origin of the other half, the C-1"-C-13" moiety of 1. It is likely that this part again is formed from a cinnamoyl-CoA and malonate-derived C₂ units. This time two units of malonate are involved in the chain elongation to form the C₆-C₅-C₄ backbone and no cyclization takes places here. Similar co-occurrence of a linear-type polyketide with chalcone and stilbene class of cyclized molecules is observed in Hydrangea macrophylla var. thunbergii. Ebizuka et al. succeeded in isolating a new member of polyketide synthase (PKS) that catalyzes only chain elongation without ring cyclization.

The known compounds, 3-hydroxy-5-methoxystilbene (2), 2',6'-dihydroxy-4'-methoxydihydrochalcone (3), stigmaster-3-ene-4-one, and β-sitosterol were identified by comparing their melting points and spectroscopic data with those described in the literature.

Compound 3 was assayed using a brine shrimp (Artemia salina Leach) lethality test to determine its bioactivity following the microwells method. The brine shrimp lethality test has been widely used as a general bioassay tool before applying other bioassays. The result of brine shrimp lethality test of 3 exhibited positive activity (LC₅₀ 7.26 µg/ml). Compound 3 has also been isolated from Piper aduncum (Piperaceae) and it showed cytotoxic activity on the KB cell of nasopharyngeal carcinoma cells (ED₅₀ >10 µg/ml) and antimicrobial activity against Bacillus subtilis and Micrococcus luteus.

Stilbene-type compounds are rare in the Lauraceae family and only one result from Lindera reflexa has been published. Compounds 1 and 2 are the first examples to be isolated from Cryptocarya genus.
Experimental

$^1$H- and $^{13}$C-NMR spectra were recorded on a JEOL JNM GSX500A. Proton spectra were measured at 500 MHz and carbon spectra at 125 MHz (TMS as an internal standard). UV spectra were recorded on a JASCO V-560 model UV spectrometer. IR spectra were measured on a JASCO FT/IR 230 IR spectrometer. Mass spectra were obtained by use of a JEOL JMS-AM20 (EI/MS) or a JEOL JMS-HX-110A (FABMS, HR-FABMS) mass spectrometer. Chromium spectra were recorded on a JASCO J-720WII instrument. For liquid chromatography, a silica gel packed column (Kusano CPK-HS-221-S) was used. Column chromatography was carried out by use of Merck silica gel 60 (70–230 mesh) and 230–400 mesh (for flash chromatography). For TLC Merck silica gel 60 F$_{254}$ plates (0.25 mm thick) were used.

Plant Material

Stem bark of Cryptocarya idenburgenesis Allen. was extracted from Putaluy village, Kecamatan Karulur, Kabupaten Jaya Wi-jaya, Irian Jaya Island, Indonesia. A herbarium specimen was deposited at the Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

Extraction and Isolation

The dried-milled stem bark of C. idenburgen-sis (2.5 kg) was percolated with $n$-hexane, then with MeOH to give the $n$-hexane extract (3.43 g) and MeOH extract (206 g). The MeOH extract was then partitioned with CHCl$_3$ to afford a CHCl$_3$ extract (41.0 g). Separation of the CHCl$_3$ extract used column chromatography on silica gel with $n$-hexane, EtOAc, and then subjected to silica gel flash column chromatography, MPLC and followed by preparative TLC to give a new compound idenburgen by a yellow viscous solid (1, 10.7 mg), 3-hydroxy-4-methoxyisobenzene (2, 47.5 mg), and 2,6'-dihydroxy-4'-methoxydihydrochalcone (3, 36.4 mg). On the other hand, the $n$-hexane extract was separated using silica gel chromato-graphy with solvent $n$-hexane-benzene-CHCl$_3$-methanol. Purification of two major fractions by flash column chromatography yielded stigmast-4-en-3-one (80.3 mg) and β-sitosterol (18.6 mg).

Idenburgenone (1): Yellowish viscous solid. $R_f$ value 0.63 (SiO$_2$, solvent system: 50% EtOAc-$n$-hexane). $^1$H-NMR see Table 1. $^{13}$C-NMR see Table 2. IR (KBr) cm$^{-1}$: 742, 782, 1049, 1112, 1213, 1725, 2931, and 3019. UV $A_{	ext{max}}$ (MeOH) nm (log $e$): 203 (3.77), 241 (3.53), 254 sh (3.47), 308 (3.45), 318 (3.43), 330 sh (3.26). CD (MeOH, $c$ 0.54×10$^{-3}$, 18°C) nm (Δε): 205.6 (+1.9), 212.6 (0), 220.8 (−3.0), 235.4 (−0.7), 242.2 (0), 265.6 (−0.5), 272.6 (0), 298.6 (−1.3). EI-MS m/z: 442 (M$^+$, 28%), 341 (100%). HR-FABMS (NBA) m/z: 442.2126 M$^+$. (Calcd for C$_{28}$H$_{38}$O$_{4}$: 442.2145)

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