Studies on the Constituents of Fruits of *Helicteres isora* L. 1)

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Three new compounds; 4′-O-β-D-glucopyranosyl rosmarinic acid (2), 4,4′-di-β-D-glucopyranosyl rosmarinic acid (3) and 2R-O-(4′-O-β-D-glucopyranosyl caffeyl)-3-(4-hydroxyphenyl) lactic acid (4) were isolated together with rosmarinic acid (1) from the fruit of *Helicteres isora* L. (Sterculiaceae), an Indonesian medicinal plant. The structures of these compounds, including the absolute stereochemistry of 4, were elucidated by spectroscopic analysis and chemical means. Compound 3 had greater scavenging activity against superoxide anion produced with xanthine and xanthine oxidase (XOD) than rosmarinic acid (1).

Key words *Helicteres isora*; isorinic acid; rosmarinic acid glycoside; rosmarinic acid; Sterculiaceae

As a continuation of our studies on Jamu and the medicinal resources in Indonesia, we have isolated three new compounds: 4′-O-β-D-glucopyranosyl rosmarinic acid (2), 4,4′-O-di-β-D-glucopyranosyl rosmarinic acid (3) and 2R-O-(4′-O-β-D-glucopyranosyl caffeyl)-3-(4-hydroxyphenyl) lactic acid (4), together with rosmarinic acid (1) from the fruit of *Helicteres isora* L. *H. isora* (Sterculiaceae) is one of the Jamu raw materials used in traditional folk medicine in Indonesia; it is called “Buah Kayu Ules or Ulet-Ulet” on Java and an anomeric proton signal [d 4.77 (1H, d, J=7.3 Hz)] and an anomeric carbon signal (δ 101.65). Enzyme hydrolysis (emulsin) of 2 yielded rosmarinic acid (1), [α]D +74.4° (c=0.5, MeOH) and 4′-O-(4-glucopyranosyl rosmarinic acid) (3) having the absolute configuration (R) at C-8 from optical rotation.

Compound 2 was isolated as a colorless amorphous powder, [α]D +9.0° (c=0.9, MeOH), molecular formula C24H22O13, from negative ion HR-FAB-MS. The anthrone reaction was positive, suggesting for 2 to be a glycoside. The 1H- and 13C-NMR spectra of 2 were similar to those of 1, besides signals corresponding to the sugar moiety which included an anomeric proton signal [δ 4.77 (1H, d, J=7.3 Hz)] and an anomeric carbon signal (δ 101.65). Enzyme hydrolysis (emulsin) of 2 yielded rosmarinic acid (1), [α]D +74.4° (c=0.5, MeOH) and 4′-O-(4-glucopyranosyl rosmarinic acid) (3) having the absolute configuration (R) at C-8 from optical rotation.

Compound 3 was obtained as a colorless amorphous powder, [α]D −17.9° (c=1.0, H2O) and had a molecular formula C29H26O18 from negative ion HR-FAB-MS. The 1H- and 13C-NMR spectra showed two anomeric proton signals [δ 4.77 (1H, d, J=7.3 Hz), 4.61 (1H, d, J=7.1 Hz)], two anomic

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carbon signals (δ 101.68, 102.56) and characteristic carbon signals due to two glucopyranosyl moieties together with proton and carbon signals corresponding to rosmarinic acid. The enzymic hydrolysis (emulsin) of 3 yielded rosmarinic acid (1), [α]Dp +74.8° (c=1.0, MeOH) and only 1-glucose as the sugar moiety. In the HMBC spectrum of 3, the anomeric proton signals at δ 4.77 and δ 4.61 exhibited cross peaks due to long-range coupling with the carbon signals at δ 146.56 (C-4) and δ 147.47 (C-4′), respectively. Furthermore, in a NOESY experiment, NOE correlations were observed between the anomeric proton at δ 4.77 and an aromatic proton at δ 6.98 (1H, d, J=8.3 Hz, H-5), and the anomeric proton at δ 4.61 and an aromatic proton at δ 7.10 (1H, d, J=8.5 Hz, H-5′). These findings and the coupling constants of each anomeric proton signal indicated that each α-glucose was linked to hydroxyl groups at C-4 and C-4′ in a β-configuration. Consequently, compound 3 was determined to be 4,4′-O-di-β-D-glucopyranosyl rosmarinic acid.

Compound 4, a colorless amorphous powder, [α]Dp = -16.0° (c=0.3, H2O), had a molecular formula C24H26O12 from negative ion HR-FAB-MS. In the comparison of the 1H- and 13C-NMR spectra of 4 with those of 2, compound 4 showed NMR signals similar to those of 2, except for the proton and carbon signals corresponding to the 4-hydroxyphenyl group instead of those due to the 3,4-dihydroxyphenyl group in 2. In the correlation spectroscopy via long range coupling (COLOC) experiments of 4 described in Fig. 2, long range correlations were observed between the aromatic carbon (C-1) at δ 127.32 and both the oxygen-bearing methine proton (H-8) at δ 5.02 and the aromatic protons (H-3, H-5) at δ 6.67, and the aromatic carbon (C-1′) at δ 128.56 and both the olefinic proton (H-8′) at δ 6.40 and the aromatic proton (H-5′) at δ 7.12. The enzymic hydrolysis (emulsin) of 4 yielded α-glucose and compound 5 (C18H16O7). On the basis of these findings, the planar structure of 5 was elucidated as 2-O-caffeoyl-3-(4-hydroxyphenyl) lactic acid. The absolute configuration of 5 was deduced by the following manner. The saponification of compound 5 followed by acidification furnished caffeic acid and 2R-3-(p-hydroxyphenyl)-lactic acid [6, [α]Dp +13.9° (c=0.6, MeOH)]. The stereochemistry of 6 was supported by the phenylglycine methyl ester (PGME) method. The values of ∆δ [δ(S)-PGME amide–δ(R)-PGME amide] in the 1H-NMR spectra (Fig. 3) suggested that the absolute configuration at C-2 of 6 was (R). The α-glucose was indicated to be linked to the hydroxyl groups at C-4′ from...
the NOE observed between the anomic proton (1H) and the aromatic proton (5H) and the glycosidic linkage was β-
configuration from the coupling constant (J = 7.3 Hz).

Accordingly, compound 4 was established as 2R-O-(4’-O-β-
glucopyranosyl caffeoyl)-3-(4-hydroxyphenyl) lactic acid.

We named compound 5 as isorinic acid. Diglucosyl ros-
marinic acid (3, 51.5%, 0.1 mm) exhibited greater scavenging
activity against superoxide anion produced with xanthine and
xanthine oxidase (XOD) than rosmarinic acid (1, 35.5%, 0.1
mm), when superoxide dismutase (SOD) was used as a posi-
tive control (48.0%).

Experimental

General Procedures The following instruments were used to obtain
physical data: melting point, Yanagimoto micro-melting point apparatus
(values are uncorrected); optical rotation, JASCO DIP-1000 Digital
polarimeter; IR spectra, Shimazu FT-IR 8300 infrared spectrometer; UV spec-
tra, Hitachi U-3000 spectrophotometer; HR-FAB-MS, JEOL JMS-BU20
spectrometer using diethanolamine as a matrix. 1H-NMR spectra, Bruker
DPX-400 FT-NMR spectrometer (400 MHz) with tetramethylsilane as an
internal standard.

The 1H- and 13C-NMR spectra: See Tables 1, 2.

Condensation of Compound 6 with β-Glucosidase (Emulsinu) A mix-
ture of compound 2 (30 mg) and β-glucosidase (40 mg) in AcOH–AcOA
buffer (pH 4.7, 5 ml) was incubated with gentle stirring at 37°C for 20 h.
The reaction mixture was diluted with H2O (20 ml), and extracted
with EtOAc (50 ml×3). The organic layer was washed with aq sat.NaCl,
then dried, and evaporated to dryness, which was chromatographed on sephadex
 LH-20 (MeOH) to give rosmarinic acid (1, 9.2 mg, [α]23.4 +74.4° (c 0.5,
MeOH)). The aqueous layer was evaporated to dryness and the residue was
chromatographed (MPLC on silica gel (EtOAc: MeOH: H2O 85 : 10 : 5) to
yield a syrup [9.3 mg, [α]23.4 +9.0°]. The syrup was treated with (S)
PGME amide, [1]446 V oI. 47, No. 10

Found: 359.0767 (M–H). UV λmax (MeOH) nm (log ε): 216.7 (4.25),
291.0 (4.08), 329.3 (4.19). IR (KBr) cm⁻¹: 3360 (OH), 1700 (C=C), 1650,
978 (trans C=C), 1606, 1524, 1448 (aromatic ring). The 1H- and 13C-NMR
spectra: See Tables 1, 2. This product was identical with an authentic sample
(rosmarinic acid) on direct comparison (TLC, IR, 1H-NMR, 13C-NMR,
[α]190°) 3)

Compound 2: A colorless amorphous powder, [α]23.4 +9.0° (c 0.9, MeOH),
negative ion HR-FAB-MS m/z: Calcd for C18H16O8: [M-16]⁻ 582.0962,
[α]190° 3) Found: 582.0974 (M-16). UV λmax (MeOH) nm (log ε): 233.4 (4.52),
287.0 (4.22), 319.0 (4.11). IR (KBr) cm⁻¹: 3337 (OH), 1687 (C=O), 1630,
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above to afford the (R)-PGME amide. \(^1\)H-NMR (\(\delta\), CD\(_{3}\)OD): 6.99 (2H, d, \(J=8.6\) Hz), 6.62 (2H, d, \(J=8.6\) Hz), 4.27 (1H, dd, \(J=4.1, 6.8\) Hz), 2.94 (1H, dd, \(J=4.1, 14.3\) Hz), 2.77 (1H, dd, \(J=6.8, 14.3\) Hz).

**Assay for Superoxide Scavenging Activity** Superoxide scavenging activity was made by the reported method\(^9\) modified as follows: A mixture (0.5 ml) consisting of an aqueous solution of xanthine (0.5 mM, 200 \(\mu\)l), hydroxylamine \(-\)sulfonic acid (1 mg/ml, 100 \(\mu\)l) and buffer solution (final concentration of 13 mM KH\(_2\)PO\(_4\), 7 mM Na\(_2\)B\(_4\)O\(_7\) and 0.1 mM EDTA-2Na) was added to the sample solution or H\(_2\)O (100 \(\mu\)l). Then, XOD solution (6.2 \(\times\) 10\(^{-2}\) U/ml, 200 \(\mu\)l) or H\(_2\)O was added to the mixture, and the solution was incubated for 30 min at 37 °C. A solution (2.0 ml) of sulfanilic acid (3 mM), \(N\)-1-naphthylethylenediamine (30 mM), acetic acid (25%) was added to the reaction mixture. The final mixture was allowed to stand for 30 min at room temperature, and the absorption of the reaction solution at 550 nm was measured. SOD from bovine erythrocytes was used as a positive control (final concentration 0.01 mg/ml, 4.4 \(\times\) 10\(^{-2}\) U/ml). The percentage inhibition of superoxide anion is 35.5% for rosmarinic acid (1, 0.1 mM), 51.5% for compound 3 (0.1 mM) and 48.0% for SOD as a positive control, respectively.

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**References and Notes**


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