Communications to the Editor

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INHIBITORS OF PROSTAGLANDIN BIOSYNTHESIS FROM GINGER

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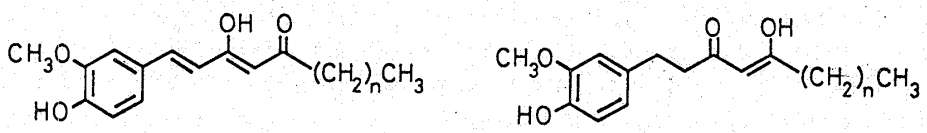
In addition to [6]-gingerol four new constituents were isolated as potent inhibitors of prostaglandin (PG) biosynthesis from ginger, the roots of *Zingiber officinale* Roscoe. Their structures were identified as [6]- and [10]-dehydrogingerdione (I and II) and [6]- and [10]-gingerdione (III and IV) by the interpretation of spectral data.

KEYWORDS——Zingiberaceae; *Zingiber officinale*; ginger; inhibitor; gingerol; dehydrogingerdione; gingerdione; prostaglandin; biosynthesis

In the course of our screening works to find biologically active principles contained in medicinal plants used in traditional medicines,[1] several medicinal plants belonging to Zingiberaceae were found to contain inhibitors of prostaglandin (PG) biosynthesis. Following this finding, extensive investigations have been carried out to isolate and identify the inhibitors. This communication mainly deals with the results of studies on the inhibitors of PG biosynthesis contained in ginger, the roots of *Zingiber officinale* Roscoe (Zingiberaceae).

Commercial fresh ginger purchased in the Japanese market was extracted with n-hexane and methanol successively. The methanol extracts were further fractionated into n-hexane-soluble and ethylacetate-soluble fractions by the usual procedure. The n-hexane-soluble fraction was chromatographed on silica gel and the obtained fractions were monitored by TLC and by their inhibitory effect on PG synthetase.[2] Fractions showing inhibition were further chromatographed on a reverse phase Lobar column (Lichroprep RP-8) to give compounds I, II, III and IV. After chromatographic purification with the guidance of the inhibitory effect, the ethylacetate-soluble fraction gave [6]-gingerol which was identified by direct comparison with an authentic sample.

Compound I, yellow needles, mp 83.5-84.5°C, $C_{17}H_{22}O_4$ (*Anal.* Calcd: C,70.32; H, 7.64. Found: C, 70.31; H, 7.71), MS m/z: 290 (M^+), 177 (base peak), IRν_{max}^{KBr} cm^{-1}: 3320 (OH), 1625 (ketone), showed UV absorption maximum (EtOH) at 374 nm (log ε=4.46). The ^{1}H-NMR spectrum ($CDCl_3$, δ ppm) of I showed the aromatic proton signals of a 1,2,4-substituted benzene ring (6.9-7.2, 3H), signals of olefinic



I : [6]-dehydrogingerdione n=4
II : [10]-dehydrogingerdione n=8
III : [6]-gingerdione n=4
IV : [10]-gingerdione n=8

Chart 1

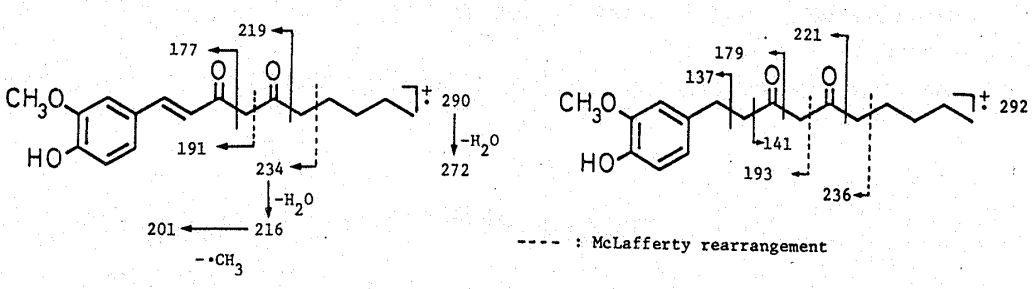


Chart 2

protons of a *trans*-double bond (6.35, 1H, d, J=16Hz and 7.52, 1H, d, J=16Hz), a methoxyl signal (3.94, 3H, s), signals of methylene groups (2.83, 2H, m, 1.68, 2H, m, 1.36, 4H, m) and a methyl signal (0.93, 3H, t, J=7Hz). On addition of D_2O, two broad singlets at δ 5.86 (1H) and δ 15.0 (1H) disappeared immediately, while the signal intensity of the singlet at δ 5.62 (1H) decreased slowly. The spectral data mentioned above clearly indicated that compound I was [6]-dehydrogingerdione (I) which was recently synthesized by Whiting *et al.*[3] The melting point reported for [6]-dehydrogingerdione was in good agreement with that of compound I.

Compound II, yellow needles, mp 69-69.5°C, $C_{21}H_{30}O_4$ (M^+: m/z 346.2154, Calcd: 346.2142), MS m/z: 346 (M^+, 15%), 328 (4%), 219 (18%), 216 (39%), 201 (15%), 191 (42%), 177 (100%), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1625 (ketone), UV λ_{max}^{EtOH} nm (log ε): 374 (4.44), showed spectral data very similar to those of I. The ^{1}H-NMR spectrum ($CDCl_3$) of II closely resembled that of I except that the signal intensity of the methylene group at δ 1.26 corresponded to twelve protons. On the basis of these spectral data, compound II was identified as [10]-dehydrogingerdione (II).

Compound III, a colourless oil, MS m/z: 292 (M^+), 137 (base peak), $C_{17}H_{24}O_4$ (M^+: m/z 292.1646, Calcd: 292.1673). The ^{1}H-NMR spectrum ($CDCl_3$, δ ppm) of III showed aromatic proton signals of a 1,2,4-substituted benzene ring (6.6-6.9, 3H), a signal of an olefinic proton (5.43, 1H, s: intensity decreased slowly on addition of D_2O), a methoxyl signal (3.85, 3H, s), a signal of benzyl protons (2.84, 2H m), an α-proton signal of ketone (2.55, 2H, m), signals of methylene groups (1.30, 4H, m, 1.60, 2H, m, 2.27, 2H, m) and a methyl signal (0.80, 3H, t, J=7Hz). The ^{1}H-NMR and MS, especially the mass fragment at m/z 137 ($C_8H_9O_2$), suggested compound III to be a dihydroderivertive of I, [6]-gingerdione (III). The

assignment of the fragment peaks in the MS of compound III is shown in chart 2.

Compound IV, white solid, $C_{21}H_{32}O_4$ (M^+: m/z 348.2293, Calcd: 348.2299), MS m/z: 348 (M^+, 17%), 236 (2%), 221 (2%), 197 (4%), 193 (3%), 179 (4%), 150 (16%), 137 (100%), IR ν_{max}^{Cap} cm^{-1}: 3390 (OH), 1610 (ketone), showed a ^{1}H-NMR spectrum very similar to that of III except for the increase of methylene signal intensity by an amount corresponding to eight protons. These spectral data suggest that compound IV is [10]-gingerdione (IV).

IC_{50} values (50% inhibitory concentration) of [6]-gingerol, I, II, III and IV against PG synthetase were 5.5, 1.0, 2.3, 1.6 and 1.0 μM, respectively.[4] Compounds I-IV were more potent inhibitors of PG biosynthesis than indomethacin, which is known as one of the strongest inhibitors with an IC_{50} of 4.9 μM under the same assay conditions.

Whiting *et al.* proposed a biosynthetic scheme for [6]-gingerol (Chart 3)

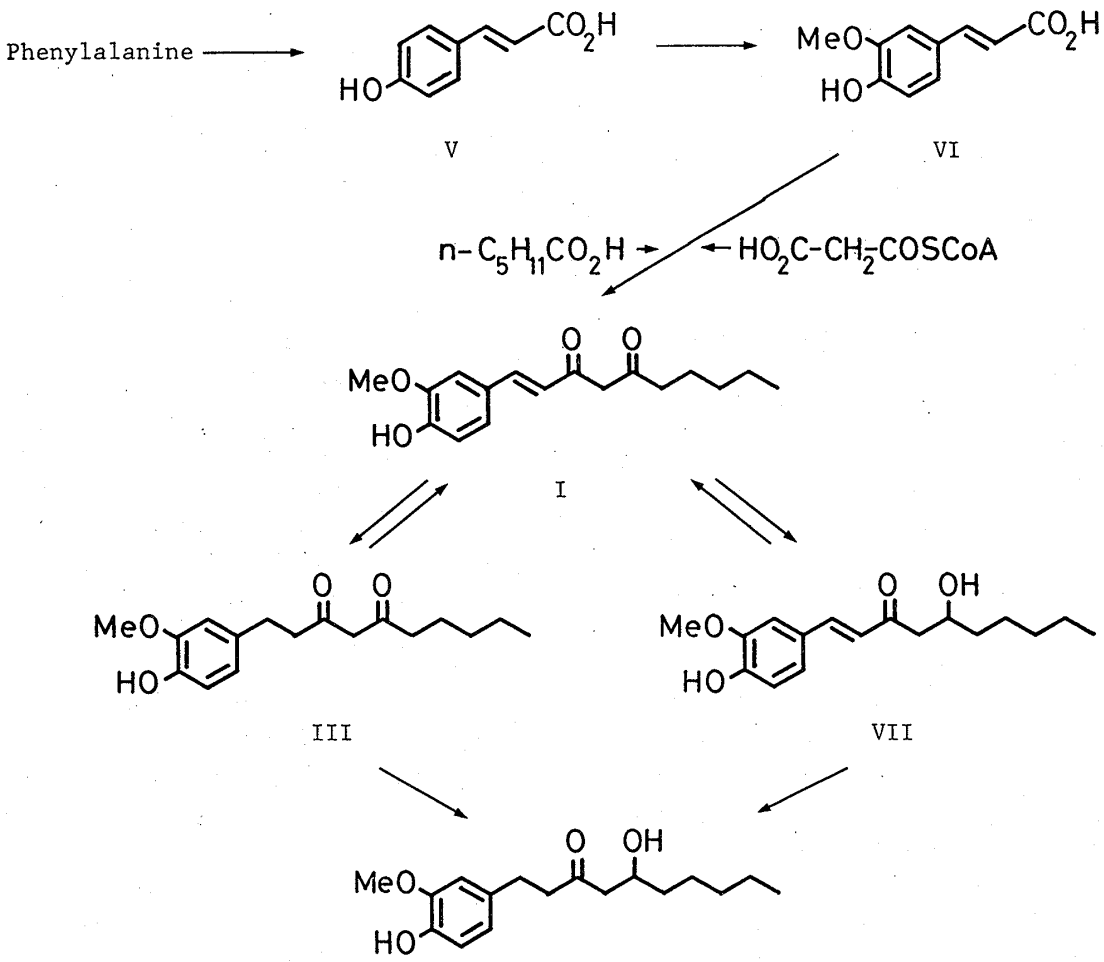


Chart 3

based on the incorporation experiments with several radio-active precursors.[5,6] They synthesized I, III and VII as hypothetical intermediates and demonstrated that they were incorporated into [6]-gingerol.[5b] However, these compounds have not previously been isolated from natural sources. The isolation of I and III from ginger along with II and IV gives strong support to the biosynthetic scheme of gingerols shown in chart 3.

REFERENCES AND NOTES

1) A part of this work was presented at the Annual Meeting of the Japanese Society of Pharmacognosy, Nagoya, Sept. 1980, and Tokyo, Oct. 1981.
2) Rabbit renal medulla microsomal fraction was used as the enzyme preparation. M. Shwartzman, Y. Gafni and A. Raz, Eur. J. Biochem., 64, 527 (1976); H. Tai, C. Tai and C. Hollander, Biochem. J., 154, 257 (1976); G. J. Blackwell, R. J. Flower and J. R. Vane, Biochim. Biophys. Acta, 398, 178 (1975).
3) P. Denniff, I. Macleod and D. A. Whiting, J. Chem. Soc., Perkin I, 1981, 82.
4) Details of assay methods and the inhibitory activity of other constituents of ginger against PG synthetase will be reported elsewhere.
5) a) P. Denniff and D. A. Whiting, J. Chem. Soc., Chem. Comm., 1976, 711; b) I. Macleod and D. A. Whiting, J. Chem. Soc., Chem. Comm., 1979, 1152.
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