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Studies on the Antitumor Bisabolane Sesquiterpenoids Isolated from *Curcuma xanthorrhiza*

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Four bisabolane sesquiterpenoids, α-curcumene, *ar*-turmerone, β-atlantone and xanthorrhizol, were isolated as major antitumor constituents (against Sarcoma 180 ascites in mice) from the rhizomes of *Curcuma xanthorrhiza*. Their structures were deduced from spectroscopic evidence. The antitumor effectiveness was rated (+ + +) for α-curcumene, (+ +) for *ar*-turmerone and (+ +) for xanthorrhizol at 50 mg/kg in the total packed cell volume method.

Keywords——*Curcuma xanthorrhiza*; Zingiberaceae; antitumor substance isolation; antitumor activity; bisabolane; sesquiterpenoid; α-curcumene; *ar*-turmerone; xanthorrhizol

We have been carrying out preliminary antitumor screening tests of crude drugs and collected plants[1,2] with Sarcoma 180 ascites in mice.[3] Recently, 60 species of Indonesian medicinal plants were subjected to the antitumor screening test. It became apparent that Temu Lawak, *Curcuma xanthorrhiza* (Zingiberaceae), which is utilized as a tonic in Indonesia and as a choleretic drug in Europe, showed a significant effect against Sarcoma 180 ascites in mice. In this paper, we report on the isolation and structural elucidation of the antitumor substances in *C. xanthorrhiza*.

When an aqueous solution of the methanolic extract prepared from the rhizomes of *C. xanthorrhiza* was partitioned successively with *n*-hexane, chloroform and *n*-butanol as shown in Chart 1, the antitumor activity against Sarcoma 180 ascites in mice was concentrated in the *n*-hexane extract. The extract was subjected to column chromatography on silica gel and was eluted with *n*-hexane, benzene, benzene–chloroform (1 : 1), chloroform and chloroform–methanol (1 : 1) successively. When each fraction was subjected to bio-assay against Sarcoma 180 ascites in mice, both the *n*-hexane and benzene fractions exhibited strong antitumor activity. Both the active fractions were independently re-chromatographed over silica gel, and the eluates were fractionated with the guidance of bio-assay against Sarcoma 180 ascites in mice as shown in Chart 1. As can be seen from Chart 1, compounds I, II, III and IV were isolated as major substances of the antitumor active fractions.

Compound I, a colorless oil, had the molecular formula $C_{15}H_{22}$ from the high-resolution mass spectrum (MS) (*m/z*: 202.1754 (M^+), Calcd 202.1720). On the basis of the signals due to a 1,4-substituted benzene ring (7.08, s), four methyl groups (1.22, d, $J=7$ Hz; 1.53, 1.69 and 2.32, s, respectively) and one olefinic proton (5.12, t, $J=7$ Hz) in the proton nuclear magnetic resonance (^{1}H-NMR: $CDCl_3$, δ, ppm) spectrum, compound I was assumed to be α-curcumene. The signals in the ^{13}C-NMR spectrum could be reasonably assigned as indicated in Table I, and other data were compatible with the literature values.[4,5] Compound II, a colorless oil, MS *m/z*: 216 (M^+), showed α,β-unsaturated carbonyl group absorptions (1690 and 1620 cm^{-1}) in the infrared (IR) spectrum. When the ^{13}C-NMR spectrum of compound II was compared with that of compound I, the C_9 signal of compound I at δ 26.20 (t) was found to be shifted downfield to δ 199.55 (s) in compound II. From the above evidence, compound II

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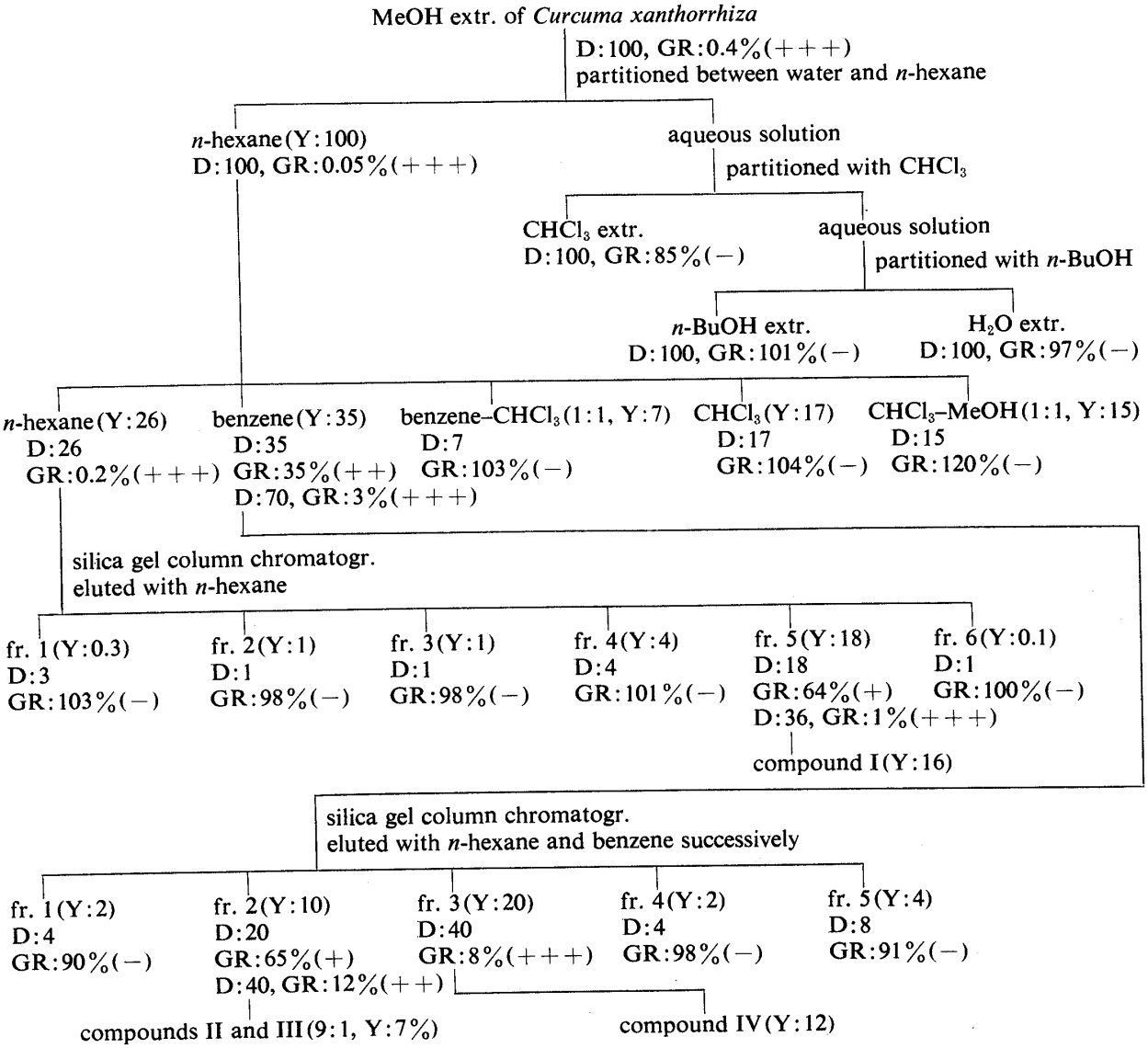

Chart 1. Antitumor Activity of Fractions Isolated from *C. xanthorrhiza*

1) Antitumor activity was determined against Sarcoma 180 ascites in mice and the effectiveness was evaluated by the total packed cell volume method. Sarcoma 180 was implanted *i.p.* (1×10^6) in ICR mice ($n=6$) on day 0.
2) Y means yield (%) from the *n*-hexane extract. D means dose (mg/kg/d); drugs were given daily at the indicated doses (*i.p.*) for 5 consecutive days (1—5 d). GR means growth ratio; 0—10% (+++), 11—40% (++), 41—65% (+), over 66% (−).
3) In the final purification of compounds I—IV, HPLC was carried out on a CIG column system with 0.05 mm silica gel.

was concluded to be *ar*-turmerone.[6-8] Compound III, a colorless oil, high MS *m/z*: 218.1687, Calcd 218.1670 for $C_{15}H_{22}O$, showed conjugated carbonyl group (1685 and 1620 cm^{-1}) and *exo*-methylene (3100, 1640 and 890 cm^{-1}) absorptions in the IR spectrum. The presence of an *exo*-methylene was also supported by the signals at δ 4.86, 4.97 (1H, d, $J=1$ Hz, respectively) and 112.14 (t) in the ^{1}H- and ^{13}C-NMR spectra. Finally, compound III was concluded to be β-atlantone[9] from the various spectral data. Compound IV, a colorless oil, MS *m/z*: 218 (M^+) and IR (CCl_4): 3620 cm^{-1}, was indicated to be 2- or 3-substituted hydroxy-α-curcumene from the aromatic proton signals ($CDCl_3$, δ, ppm: 6.59 (1H, d, $J=2$ Hz), 6.67 (1H, dd, $J=2$ and 8 Hz) and 7.01 (1H, d, $J=8$ Hz)) in the ^{1}H-NMR spectrum. Compound IV was found to contain a 1,3,4-substituted benzene ring system as shown in Fig. 1 by comparing the chemical

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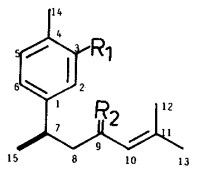
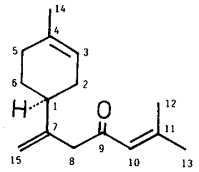


I: $R_1=H$, $R_2=H_2$
II: $R_1=H$, $R_2=O$
IV: $R_1=OH$, $R_2=H_2$



III

Fig. 1. Structures of Bisabolanes from *C. xanthorrhiza*

TABLE I. ^{13}C Chemical Shifts of Bisabolanes from *Curcuma xanthorrhiza*

Carbon No.	Compounds			
	I	II	III	IV
1	144.66 (s)	143.57 (s)	39.78 (d)	147.08 (s)
2	126.90 (d)	126.56 (d)	30.50 (t)[a]	113.47 (d)
3	128.95 (d)	128.98 (d)	120.33 (d)	153.48 (s)
4	135.13 (s)	135.32 (s)	133.65 (s)	120.85 (s)
5	128.95 (d)	128.98 (d)	31.08 (t)[a]	130.65 (d)
6	126.90 (d)	126.56 (d)	27.96 (t)	119.35 (d)
7	39.04 (d)	35.23 (d)	148.12 (s)	38.98 (d)
8	38.48 (t)	52.64 (t)	51.09 (t)	38.34 (t)
9	26.20 (t)	199.55 (s)	198.74 (s)	26.12 (t)
10	124.59 (d)	124.02 (d)	123.04 (d)	124.43 (d)
11	131.31 (s)	154.75 (s)	155.68 (s)	131.29 (s)
12	17.66 (q)	20.58 (q)	20.76 (q)	15.28 (q)
13	25.69 (q)	27.50 (q)	27.68 (q)	25.66 (q)
14	20.97 (q)	20.93 (q)	23.35 (q)	18.64 (q)
15	22.45 (q)	21.97 (q)	112.14 (t)	22.31 (q)

The measurements were made on a JEOL FX-200 spectrometer in $CDCl_3$ with TMS as an internal reference and are expressed in terms of ppm. *a*) The assignments may be reversed.

TABLE II. Antitumor Activity Tests on Sarcoma 180 Ascites in Mice

Compound	Dose (mg/kg)	Mice (ICR)	BWC	PCV/TV	GR (%)	Assessment
I	10	6	+4.2	0.31	97.2	−
	20	6	+2.1	0.16	31.0	++
	50	6	+2.4	0.00	0.0	+++
II	25	6	+3.1	0.39	77.2	−
	50	6	+2.7	0.12	22.2	++
IV	50	6	+3.6	0.13	17.7	++

The effectiveness was evaluated by means of the total packed cell volume method. PCV, packed cell volume; TV, total volume; GR, growth ratio = PCV (test groups)/PCV (control groups) × 100; BWC, body weight change = (day 7 weight − TV)/day 0 weight.

shifts of the carbon-13 NMR signals due to the aromatic ring moiety with those calculated from the empirical substituent increments in benzene derivatives.[10] Therefore, compound IV was concluded to be xanthorrizol.[11]

Next, the antitumor activity of the isolated bisabolane sesquiterpenoids against Sarcoma 180 ascites in mice was evaluated by the total packed cell volume method.[3] The results are

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shown in Table II. β-Atlantone (III) could not be tested because of the very small amount of sample obtained from the original plant. As can be seen from Table II, α-curcumene (I) showed a dose-dependent effect: (−) at 10 mg/kg, (++) at 20 mg/kg and (+++) at 50 mg/kg. *ar*-Turmerone (II) and xanthorrhizol (IV) showed lower activity (++) at 50 mg/kg than I. On the other hand, I showed no significant activity against P388 lymphocytic leukemia in mice, in the dose range of 50 to 200 mg/kg.

Recently, the antitumor activity of β-elemene obtained from *Curcuma aromatica*[12-14] has been reported. Curcuma essential oil containing these compounds is clinically applied to carcinoma colli in China.[12] From this viewpoint, it is interesting that these bisabolane sesquiterpenoids showed antitumor activity in our experimental system.

Experimental

Silica gel column chromatography was carried out on Wakogel C-200 (100—200 mesh). In general, silica gel for column chromatography was employed at amounts equivalent to 50—100 times the sample amount. For final purification, high-performance liquid chromatography (HPLC) was carried out on a CIG column system (Kusano Scientific Co., Tokyo) with Wakogel LC-50H (0.05 mm silica gel) as the stationary phase. Spectral data were obtained on the following machines; ultraviolet (UV) on a Hitachi 557, NMR on a JEOL FX-200 or a Varian EM 390, high- and low-resolution MS on a Hitachi M-80 and IR on a JASCO A-302. An Ohkura GC-103 gas chromatograph equipped with a flame ionization detector was employed for analysis.

Extraction and Isolation——The rhizomes of *Curcuma xanthorrhiza* used in this experiment were purchased in Indonesia. The crude drug (800 g) was extracted with methanol (2.5 l) three times. The concentrated methanolic extract (113 g) was diluted with water and then shaken successively with *n*-hexane, chloroform and *n*-butanol in a separatory funnel three times. The three portions of each organic solvent were combined and concentrated *in vacuo*. The *n*-hexane extract, which showed significant activity against Sarcoma 180 ascites in mice, was fractionated as shown in Chart 1. When thin layer chromatography (TLC) was done on 0.25 mm silica gel plates ($60F_{254}$, Merck) with *n*-hexane–AcOEt (19 : 1), the *Rf* values were 0.66 for α-curcumene (I), 0.25 for *ar*-turmerone (II), 0.28 for β-atlantone (III) and 0.11 for xanthorrhizol (IV).

Compound I (α-Curcumene): Colorless oil. $[\alpha]_D^{17}$ −43.7° (c=0.37, $CHCl_3$). High MS m/z: M^+ 202.1716, Calcd 202.1754 for $C_{15}H_{22}$. Low MS m/z (%): 202 (M^+, 41), 132 (79), 119 (100), 105 (47), 91 (22). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1520 (aromatic C=C). UV λ_{max}^{EtOH} nm (ε): 254 (450), 258.5 (490), 264.5 (530), 273 (500). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.22 (3H, d, J=7 Hz), 1.53 (3H, s), 1.69 (3H, s), 2.32 (3H, s), 2.67 (1H, qt, J=7 and 7 Hz), 5.12 (1H, t, J=8 Hz), 7.08 (4H, s).

Compound II (*ar*-Turmerone): Colorless oil. $[\alpha]_D^{20}$ +64.5° (c=0.12, $CHCl_3$). High MS m/z: M^+ 216.1529, Calcd 216.1509. Low MS m/z (%): 216 (M^+, 32), 119 (57), 83 (100), 55 (18). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1690, 1620 (C=C–CO). UV λ_{max}^{EtOH} nm (ε): 275 (15700). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.22 (3H, d, J=7 Hz), 1.84 (3H, s), 2.10 (3H, s), 2.30 (3H, s), 2.59 (1H, dd, J=16 and 8 Hz), 2.71 (1H, dd, J=16 and 7 Hz), 3.29 (1H, q, dd, J=7, 7 and 8 Hz), 6.04 (1H, br s).

Compound III (β-Atlantone): Colorless oil. $[\alpha]_D^{26}$ −48.4° (c=0.12, EtOH). High MS m/z: M^+ 218.1687, Calcd 218.1665 for $C_{15}H_{22}O$. Low MS m/z (%): 218 (M^+, 12), 135 (24), 120 (100), 105 (32), 83 (74), 55 (22). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1685, 1620 (C=C–CO), 3100, 1640, 890 (CH_2=C). UV λ_{max}^{EtOH} nm (ε): 242 (10900). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.64 (3H, s), 1.89 (3H, s), 2.15 (3H, s), 3.13 (2H, s), 4.86 (1H, br s), 4.97 (1H, br s), 5.39 (1H, br s), 6.15 (1H, br s).

Compound IV (Xanthorrhizol): Colorless oil. $[\alpha]_D^{20}$ ±0° (c=0.20, $CHCl_3$). High MS m/z: M^+ 218.1691, Calcd 218.1665 for $C_{15}H_{22}O$. Low MS m/z (%): 218 (M^+, 42), 148 (24), 136 (100), 121 (42), 91 (14). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (OH). UV λ_{max}^{EtOH} nm (ε): 275 (1900), ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.18 (3H, d, J=7 Hz), 1.54 (3H, s), 1.68 (3H, s), 2.21 (3H, s), 2.61 (1H, qt, J=7 and 7 Hz), 4.60 (1H, s), 5.11 (1H, t, J=7 Hz), 6.59 (1H, d, J=2 Hz), 6.67 (1H, dd, J=2 and 8 Hz), 7.01 (1H, d, J=8 Hz).

Assay of Activity Against Sarcoma 180 Ascites[15]——ICR male mice, 5 weeks old, supplied by Clea Japan Co., Ltd., were used in groups of 6 animals. Sarcoma 180 ascites, provided by the National Cancer Center Research Institute and maintained in successive generations by us, was implanted *i.p.* at 1×10^6 cells/body. Administration of a test drug was started at one day after the implantation and continued for 5 d by the *i.p.* route. The effectiveness was evaluated by means of the total packed cell volume method[3]; growth ratio (GR %) = (packed cell volume (PCV) of test groups/PCV of control groups) × 100; GR = 0—10% (+++), 11—40% (++), 41—65% (+) and over 66% (−).

Assay of Activity Against P388 Lymphocytic Leukemia[15]——CDF_1 male mice, 5 weeks old, supplied by Japan Charles River Co., Ltd., were used in groups of 6—7 animals. P388 lymphocytic leukemia, provided by the Cancer Research Institute and maintained in successive generations by us, was implanted *i.p.* at 1×10^6 cells/body. A test

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drug was given *i.p.* at one day after the implantation and continued for 5 d. The effectiveness was evaluated in terms of the increase of life span (ILS, $T/C\%$).

Drug Treatment——A 0.5% solution of carboxymethyl cellulose (CMC) in isotonic sodium chloride was used as a vehicle for the injection of test drugs. The dose ranges used for treatment are shown in Chart 1 and Table II. Control group mice received equal volumes of normal saline containing 0.5% CMC. The results were evaluated according to the standard methods described above.

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