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# Isolation of Cytotoxic Compounds from the Leaves of *Xanthium strumarium* L.

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**Abstract**—MeOH extract of the leaves of *Xanthium strumarium* L. were found to have cytotoxic activities against five human tumor cell lines. Cytotoxicity-guided chromatographic fractionation led to the isolation of the $\alpha$-methylene containing sesquiterpenes, xanthatin, 8-*epi*-xanthatin and 8-*epi*-tomentosin. 8-*epi*-Xanthatin was found to be far more cytotoxic than 8-*epi*-tomentosin, which lacks the conjugated enone moiety present in 8-*epi*-xanthatin.

**Keywords**—*Xanthium strumarium* L. · Compositae · cytotoxic sesquiterpenes

As part of our continuing search for novel antitumor agents of plant origin, *Xanthium strumarium* L. was selected for study since the MeOH extract of leaves of the species was found to exhibit significant cytotoxicities against five human tumor cell lines. This plant, commonly known as 'Dokomari' in Korean, is an annual herb which grows widely throughout southern Korea. Its root is used as a bitter tonic and in the treatment of cancer and strumous disease (Chopra *et al.*, 1956). The chemistry of the species shows that xanthanolides are characteristic for the genus *Xanthium*, where these lactones have always been isolated. Plourde and Mockle (1960) have isolated xanthinin from the leaves of Canadian *X. strumarium* while Minnato and Horbe (1965) have reported the isolation of xanthumin from Japanese *X. strumarium* which is a stereoisomer of xanthinin.

The present paper deals with the isolation and identification of active principles responsible for cytotoxic activity from the MeOH extract of the leaves of this plant.

## Experimental

**General** - Mps: uncorrected; HRMS: JEOL JMS-DX 303; The UV spectra were taken in MeOH and IR spectra as KBr pellets. NMR spectra were recorded at 300 MHz ($^1$H) and 75MHz ($^{13}$C) with TMS as int. std. The column chromatography was carried on silica gel (Merck 9390). Merck silica gel 60 $PF_{254}$ was used for TLC.

**Plant material** - The leaves of *Xanthium strumarium* L. were collected at Taejon, Korea, in August 1992. The botanical identity was established by Prof. Kyong-Soon Lee, College of Pharmacy, Chungbuk National University, Cheongju. A voucher specimen of this material is deposited in our institute.

**Bioassays** - The extracts, fractions and isolated pure compounds were routinely evaluated in a test for *in vitro* cytotoxicity against human tumor cell lines of A-549, SK-OV-3, SK-MEL-2, XF-498 and HCT-15 using doxorubicin as a reference. The cytotoxic activi-

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ties against the above human tumor cell lines were determined colorimetically at 520 nm after staining viable cells with 0.4% SRB (sulphorhodamin B) soln (Skehan *et al.*, 1990).

**Extraction and isolation** - Fresh leaves of *X. strumarium* L. (2 Kg) were extracted with MeOH. The extract was partitioned between $CH_2Cl_2$ and $H_2O$. The $CH_2Cl_2$ soluble fraction was chromatographed on silica gel and elution started with a gradient of MeOH in $CH_2Cl_2$ consisting 4 steps, containing 5, 25, 50, 100% MeOH, respectively. The fraction eluted by 5% MeOH-$CH_2Cl_2$ was rechromatographed on silica gel, eluting with a gradient of EtOAc in n-hexane consisting 4 steps, containing 10, 30, 50, 100%. The fraction eluted by 50% EtOAc-n-hexane was further chromatographed by prep. TLC to yield xanthatin (1, 21mg), 8-*epi*-xanthatin (2, 153mg) and 8-*epi*-tomentosin (3, 50mg).

Xanthatin(1): Colorless needles, mp 113-5°, $^{13}C$ NMR ($CDCl_3$): $\delta$ 198.5, 169.7, 149.6, 145.1, 140.2, 138.2, 125.0, 118.4, 82.4, 49.3, 37.5, 32.4, 28.5, 28.2, 20.0; MS m/z (rel. int.): 246 $[M]^+$ (100), 231 (44), 204 (65), 175 (83), 123 (91), 109 (53).

8-*epi*-Xanthatin(2): Colorless needles, mp 71-2°, $^{13}C$ NMR ($CDCl_3$): $\delta$ 198.4, 170.0, 146.4, 143.2, 137.4, 135.3, 127.3, 123.4, 78.4, 41.3, 36.4, 31.5, 27.6, 27.3, 21.3; HRMS m/z 246.1254 ($[M]^+$, calcd for $C_{15}H_{18}O_3$ : 246.1252); MS m/z(rel. int.): 246$[M]^+$ (51), 231 (18), 203 (20), 135 (100), 122 (32), 93 (86).

8-*epi*-Tomentosin(3): Colorless liquid, $^{13}C$ NMR ($CDCl_3$): $\delta$ 208.3, 170.4, 146.7, 139.4, 122.5, 118.5, 82.3, 48.3, 42.9, 36.9, 35.3, 35.0, 30.2, 26.3, 18.3; MS m/z(rel. int.): 248$[M]^+$ (68), 230 (36), 205 (19), 190 (89), 177 (32), 149 (44), 123 (66), 95 (50).

## Results and Discussion

$CH_2Cl_2$ and water partitions of the MeOH extract of the leaves of *X. strumarium* L. were tested for cytotoxicity. The active $CH_2Cl_2$ solubles was subjected to successive chromatographic separations on silica gel column and preparative TLC to isolate the major active compounds 1 and 2 along with an inactive compound 3, directing each step with the SRB cytotoxicity assay.

Compound 1, $C_{15}H_{18}O_3$ ($[M]^+$, m/z 246), was obtained as colorless needles in poor yield (0.001%). The spectral data and mp of 1 coincide very well with those reported for xanthatin (Deuel *et al.*, 1957). Its $^1H$ NMR spetrum showed the characteristic methine signals assigned to H-7and H-8 at $\delta$ 2.52 and 4.27, respec-tively. Xanthatin was isolated by Little *et al.* (1950) from *X. pennsylvanicum* as an antibacterial constituent and by Harada *et al.* (1985) from *X. strumarium* as an anti-attaching repellent against the blue messel.

Compound 2 was isolated as colorless needles. The molecular formular of 2, $C_{15}H_{18}O_3$ (m/z 246.1254) was confirmed by high resolution EIMS and was coincident with that of 1. The close resemblance of the $^1H$ and $^{13}C$ NMR spectra of 2 to those of 1, except for the signals due to a C-7 methine ($\delta$ 3.40) and a C-8 methine ($\delta$ 4.62) led us to the assumption that 2 might be a C-8 epimer of 1. The structure of 2 was identified by comparison of its $^1H$ NMR data with those from literature (McMillan *et al.*, 1975).

Compound 3 obtained as colorless liquid, was identified as 8-*epi*-tomentosin (Bohlmann *et al.*, 1978). It gave spectral features similar to those of 2, but the EI mass spectrum showed a $[M]^+$ at m/z 248, which exceeded that of 2 by 2 amu. In addition, two olefinic protons at $\delta$ 6.10 and 6.95 were not observed in the $^1H$ NMR spectrum of 3.

The structures of these known compounds 1 - 3 were identified from published data. The published NMR assignments are, however, incomplete and a high field NMR study was therefore undertaken (Table 1).

Xanthatin (1) and the 8-*epi*-xanthatin (2) were significantly cytotoxic to human tumor cell lines of A-549 (lung adenocarcinoma), SK-

Table 1. $^1H$ NMR spectral data for compounds 1, 2 and 3 ($CDCl_3$)

| H | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 7.10 d (16.0)[a] | 6.95 d (16.3)[b] | } 2.43-2.62** |
| 3 | 6.20 d (16.0)[a] | 6.10 d (16.3)[b] | |
| 5 | 6.28 dd (8.7, 6.0) | 6.17 dd (8.9, 6.3) | 5.52 dd (8.7, 6.2) |
| 6A | 2.40 m | } 2.42-2.68 m | 2.30* |
| 6B | 2.25 m | | 2.02 m |
| 7 | 2.52 m | 3.40 m | 2.50** |
| 8 | 4.27 ddd (12.1, 8.9, 2.0) | 4.62 ddd (12.3, 8.7, 2.2) | 4.24 ddd (12.1, 8.7, 2.2) |
| 9A | 1.85 m | 1.88 br q (11.1) | 1.82 m |
| 9B | 3.10 m | 2.15 m | 2.43-2.62** |
| 10 | 2.79 m | 2.80 m | 2.30* |
| 13A | 5.47 d (2.9) | 5.54 d (3.0) | 5.45 d (2.9) |
| 13B | 6.17 d (2.9) | 6.28 d (3.0) | 6.16 d (2.9) |
| 14 | 1.15 d (7.0) | 1.15 d (7.1) | 1.14 d (7.2) |
| 15 | 2.30 s | 2.28 s | 2.20 s |

Values in parentheses are coupling constants in Hz.

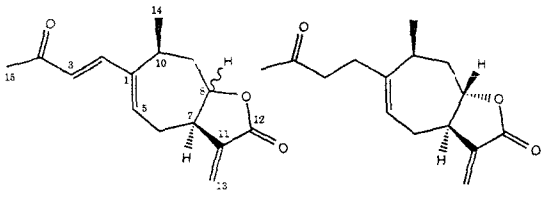
[a,b]These assignments may be reversed in each column.

*,**Not clear due to overlapping.

Table 2. Cytotoxicities of compounds 1-3 and doxorubicin(reference)

| Compounds | $ED_{50}$ ($\mu$g/ml) | | | | |
|---|---|---|---|---|---|
| | A-549 | SK-OV-3 | SK-MEL-2 | XF-498 | HCT-15 |
| 1 | 1.3 | 1.6 | 0.5 | 1.7 | 1.1 |
| 2 | 1.1 | 1.5 | 0.2 | 1.3 | 0.1 |
| 3 | >20 | >20 | >20 | >20 | >20 |
| Doxorubicin | 1.1 | 1.5 | 2.2 | 1.3 | 10.0 |

OV-3 (ovarian adenocarcinoma), SK-MEL-2 (malignant melanoma), XF-498 (CNS carcinoma) and HCT-15 (colon adenocarcinoma). However, 8-*epi*-tomentosin (3) was inactive, these facts confirming that for these compounds the $\alpha$-methylene-$\gamma$-lactone and the conjugated enone side chain contribute to cytotoxicity (Table 2) (Kupchan *et al.* 1971).



1. 8-βH
2. 8-αH

3

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