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製藥與生物技術國家型計畫年度研究成果報告

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中文摘要

從水金京之根、葉分離出具有細胞毒性之三萜化合物 Ursolic acid (**1**) 及三萜化合物 Phytol (**2**)。另外從血桐葉之醋酸乙酯可溶部份分離出具有細胞毒性之 Nymphaeol C (**3**)。上述化合物之單離純化工作是藉由 Diaion, MCI, Sephadex LH-20 及 ODS 膠體而完成。正相色層層析之進行則以矽膠體之管柱。除上述化合物外，有三十七個 pthalimide 衍生物，十四個 1,8 naphthalimide 衍生物，十四個 isoindole 衍生物，四個 pyridine 2,3 di-caboxyimide 衍生物及四個 cantharidine 衍生物已被製備。 於這些合成化合物中僅兩個 cantharidine 衍生物(**4**)及(**5**)呈現顯著之細胞毒性。

關鍵詞：抗癌，細胞毒性，水金京，血桐，Cantharidine, Ursolic acid, Phytol, Nymphaeol C

Abstract

Ursolic acid a cytotoxic triterpene (**1**) and a diterpene called Phytol (**2**) were isolated from roots and leaves of *Wendlandia formosana*. A cytotoxic flavone (-)-Nymphaeol C (**3**) was isolated from ethyl acetate soluble fraction of leaves of *Macaranga tanarius*. Isolation and purification of above compounds has been carried out by reverse phase chromatography using Diaion, MCI, Sephadex-LH 20, and ODS gels. Normal phase chromatography performed over Silica gel columns. In addition to the above, thirty seven pthalimide derivatives, fourteen 1,8 naphthalimide derivatives, fourteen isoindole derivatives, four pyridine 2,3 di-caboxyimide derivatives and four cantharidine derivatives were prepared. Among all synthetic compounds only two cantharidine derivatives (**4**) and (**5**) showed significant cytotoxic activity.

Keywords: Anticancer, Cytotoxic Activity, *Wendlandia formosana*, *Macaranga tanarius*, Cantharidine, Ursolic acid, Phytol, Nymphaeol C

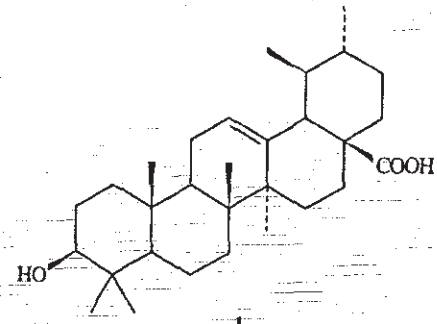
Introduction

National Health Research Institute (NHRI) has built the program project of "Studies on the active constituents of antitumor and antivirus from Formosan plants". The three years' program project will investigate the stem and root of *Wendlandia formosana*, *Macaranga tanarius* and other plants. Further, some crude extracts from 300 plants will be also prepared to provide NHRI doing high-throughput robotic screening. *Wendlandia formosana* (Rubiaceae) has been used as a folk medicine to treat a wound. The leaves of *Macaranga tanarius* (Euphoriaceae) contained hydrolyable tannin, terpenoids, and steroids. It also can cure a wound. In our previously preliminary screening of 300 and more plants, some extracts were shown activity in four kinds of cancer cell lines cytotoxic screening. Among them, the stems of ethyl acetate soluble fraction from methanol extract of *W. formosana* at 50 μg/mL was shown significant cytotoxicity in hepatoma (3 %), colon cancer (18 %), nasopharyngeal carcinoma (6 %) and gastric cancer (3 %). The root of *W. formosana* was also exhibited cytotoxicity in hepatoma (6 %), colon cancer (33 %), nasopharyngeal carcinoma (7 %) and gastric cancer (10 %). The leaves of *M. tanarius* was displayed significant cytotoxicity in hepatoma (24 %), colon cancer (36 %), nasopharyngeal carcinoma (44 %) and gastric cancer (27 %). From the above evidences, *W. formosana* and *M. tanarius* have potential for further investigation. Thus, the first year will study on the active constituents of stem from *W. formosana*. The second and third years will study of the root from *W. formosana* and the leaves from *M. tanarius*, and/or other plants.

Results and Discussion

Ursolic acid a cytotoxic triterpene (**1**) and a diterpene called Phytol (**2**) were isolated from leaves of *Wendlandia formosana*. A cytotoxic flavone, Nymphaeol C (**3**) was isolated from ethyl acetate soluble fraction of leaves of *Macaranga tanarius*. Isolation and purification of above compounds has been carried out by reversephase chromatography using Diaion, MCI, Sephadex-LH 20, and ODS gels. Normal phase chromatography performed over Si gel columns. In addition to the above, thirty seven pthalimide derivatives, fourteen 1,8

naphthalimide derivatives, fourteen isoindole derivatives, four pyridine 2,3 di-caboxyimide derivatives and four cantharidine derivatives were prepared. Among all synthetic compounds only two cantharidine derivatives (**4**) and (**5**) showed significant cytotoxic activity.



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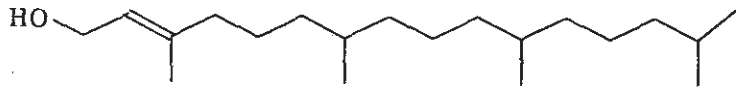

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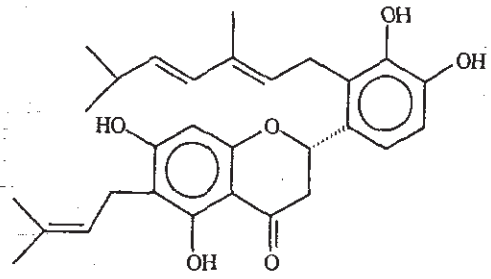

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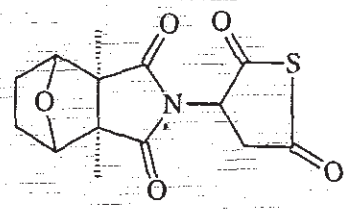

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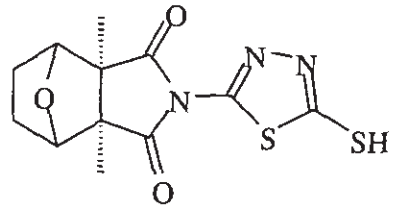

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Experimantal

Ursolic Acid (1)[1,2]: white crystalline solid, M.F. $C_{30}H_{48}O_3$. Cytotoxicity: NUGC (50 μM: 0 %; 10 μM: 99 %), HONE-1 (50 μM: 0 %; 10 μM: 102 %). ^{1}H NMR (Pyridine-d_5, 500 MHz) δ: 0.89 (s, 3H, H-25), 0.95 (d, J=6.2 Hz, 3H, H-30), 0.10 (d, J=6.3 Hz, H-29), 1.02 (s, 3H, H-26), 1.05 (s, 3H, H-24), 1.22 (s, 3H, H-27), 1.24 (s, 3H, H-23). IR (KBr): γ_{max} 3540 and 1720 cm^{-1}

Phytol (2)[3]: colourless oil, M.F. $C_{20}H_{40}O$. Cytotoxicity: NUGC (50 μM: -1%; 10 μM: 93%), HONE-1 (50 μM: 118%; 10 μM: 103%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.80 (3H, d, J=3Hz, H-18), 0.81-0.83 (m, 9H, H-17, 19 and 20), 1.48 (1H, septet, J=6.6 Hz, H-15), 1.61 (3H, s, H-16), 4.09 (2H, d, 6.8 Hz, H-1) and 5.35 (1H, hextet, J=0.8 Hz, H-2). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 16.0 (C-16), 19.6 (C-17), 19.7 (C-18), 22.5 (C-19), 22.6 (C-20), 24.4 (C-9), 24.7 (C-13), 25.0 (C-5), 27.9 (C-15), 32.6 (C-7), 32.7 (C-11), 36.6 (C-6), 37.2 (C-12), 37.3 (C-8), 37.4 (C-10), 39.3 (C-14), 39.8 (C-4), 59.1(C-1), 123.1 (C-2), 139.7 (C-3).

(-)-Nymphaeol C[4]: Amorphous powder, NUGC (50 μM: -1 %; 10 μM: 89 %), HONE-1 (50 μM: 1 %; 10 μM: 99%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.56 (3H, s), 1.64 (3H, s), 1.72 (3H, s), 1.74 (3H, s), 1.79 (3H, s), 2.05 (m, 4H), 2.72 (1H, dd, J=2.6, 17.2 Hz), 3.08 (1H, dd, J=13.2, 17.2 Hz), 3.32 (2H, d, J=7Hz), 3.43 (2H, d, J=6.6 Hz), 3.43 (2H, d, J=6.1 Hz), 5.00 (1H, t, J=6.1Hz), 5.14 (1H, t, J=6.4 Hz), 5.22 (1H, t, J=7.0 Hz), 5.48 (1H, dd, J=2.7, 13.2 Hz), 5.82 (1H, br s), 5.94 (1H, s), 6.41 (1H, br s), 6.80 (1H, d, J=8.4 Hz), 6.94 (1H, d, J=8.4Hz), 12.33 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 16.20, 17.60, 17.75, 18.75, 25.07, 25.57, 25.71, 26.34, 39.51, 42.59, 76.22, 95.33, 102.71, 107.57, 112.90, 118.53, 121.53, 121.65, 123.77, 126.40, 128.56, 131.90, 134.25, 137.90, 142.56, 144.42, 161.19, 161.29, 163.80, 196.61.

Table 1. Cytoxic activity of polyphenols from *Macaranga tanarius**,[5]

Compound No.	Compound ID	NUGC (10μM)	NUGC (50μM)	HONE-1 (10μM)	HONE-1 (50μM)
9	**NSTPOD0001**	88%	**25%**	90%	72%
10	NSTPOD0002	104%	41%	96%	98%
11	NSTPOD0003	100%	59%	99%	80%
12	**NSTPOD0004**	103%	59%	102%	**27%**
13	**NSTPOD0005**	**55%**	**16%**	**29%**	**18%**
14	NSTPOD0006	86%	**35%**	104%	71%
15	NSTPOD0007	97%	94%	89%	78%
16	**NSTPOD0008**	96%	**38%**	99%	**29%**
17	NSTPOD0009	101%	87%	89%	98%
18	NSTPOD00010	107%	103%	96%	106%
19	NSTPOD00011	103%	90%	96%	101%
20	**NSTPOD00012**	82%	**34%**	94%	85%
21	**NSTPOD00013**	100%	**64%**	92%	**34%**

*Cytotoxic polyphenols were indicated by bold letters

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