製藥與生物技術國家型計畫辦公室
National Science and Technology Program in Pharmaceuticals and Biotechnology
國家衛生研究院　台北市 115 **南港區研究院路二段一二八號**
NHRI, 128 Yen-Chiu-Yuan Road, Sec. 2, Taipei 115, Taiwan, R. O. C.
Tel:886-2-26534401 **分機** *6115-9, 6591-2　Fax:886-2-27830085*

封面格式

製藥與生物技術國家型計畫年度研究成果報告

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※**台灣產植物之抗癌與病毒活性成分研究–**　　　　　　※
※**水金京之莖，根及血桐葉之抗癌與抗病毒活性成份研究**※
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計畫類別：□**個別型計畫**　　x **整合型計畫**

計畫編號：NSC89－2323－B－038－001－

執行期間：八十九年一月一日至八十九年九月三十日

執行單位：臺北醫學大學 藥學院系

計畫主持人：徐鳳麟

共同主持人：

請檢附以下附件供審查委員參考：

- □ 89 **年度核撥經費**
- □ 90 **年度申請補助經費**
- □ 91 **年度申請補助經費**

中　華　民　國　89 **年**　10 **月**　12 **日**

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年度研究成果報告內容

一、計畫緣起。

製藥科技為一低污染、高附加價值、跨領域科技結合的產業。 歐、美、日等各主要國家均非常重視製藥工業之發展，除了對國民健康有助益外，亦能帶來經濟上的厚利。 癌症、病毒感染所引起之疾病，這些年來隨著人類生活環境之污染以及交通工具之發達，接觸頻繁所衍生之傳染而持續增加。 同時，由這類疾病造成人類健康之危害和社會問題已不容許政府之忽視。 為探討相關之藥物發展，國內一些學者在國家衛生研究院之整合下，組成製藥與生物技術國家型計畫，並於初步之篩選過程，在 300 多種植物中，發現台灣水金京(*Wendlandia formosana*)之莖、根部及血桐(*Macaranga tanarius*)葉之甲醇萃取物具有顯著之細胞毒性，特別是在乙酸乙酯可溶部份之有效成份很值得做進一步探討。

二、計畫目的。

本子計畫為〝製藥與生物技術國家型計畫〞之一員，著重於整合國內新藥研發之人才，在國家衛生研究院之主導下配合有機合成化學、藥物化學以及其他生物活性研究相關人員，企圖以三年之期間, 致力於抗癌及抗病毒活性成份之探索, 尋求可供新藥開發之先導化合物。因此本子計畫擬於三年執行期間研究上述三種藥材之活性成份，尋求值得開發之藥用化合物。 第一年以台灣水金京(*Wendlandia formosana*)莖部之活性成分探索為主要目的。

三、執行進度，包括：

1. 研究成果。

Work done in the First year (Jan – Dec 2000):

Work done during the first year has been presented in Part **1.1, 1.2, 2, 3, 4** and Part **5** is conclusion.

(1) Studies on active constituents of stem of *Wendlandia formosana*

1.1 Studies on active constituents of stem bark of *Wendlandia formosana*

As part of "Studies on the active constituents of antitumor and antivirus from *Formosana* plants" chemical examination of stem bark of *Wendlandia formosana* was carried out first. Crude methanol extract [TMCOB-WFB-1] of stem bark did not show activity [TMCOB-WFB-1; HONE-1 92% (50μg/ml); NUGC 93% (50μg/ml)]. But, one fraction (TMCOB-WFB-122, 130mg) of ethyl acetate soluble portion of methanol extract was found to be **cytotoxic** active. [TMCOB-WFB-122; HONE-1 44% (50μg/ml), NUGC 19% (50μg/ml)]. The active fraction was further examined for its constituents and two compounds [NSTPOD00014 (WHR-P9); NUGC 98% (10μM, -1% (50μM), HONE-1 61% (10μM), 1% (50μM) and [NSTPOD00015 (WFB-P11); NUGC 10% (10μM), 97% (50μM); HONE-1 99% (10μM), 106% (50μM)] were isolated.

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The compound WFR-P9 (NSTPOD00014) was very minor constituent in stem bark of *Wendlandia formosana*. Inorder to obtain spectroscopic data, the same compound was identified and isolated from the root of *Wendlandia formosana.* Only 10 mg. of the compound was obtained from the root also. Spectroscopic data of NSTPOD00014 (WFR-P9) indicated that it is a close mixture of two steroidal ketones **1** and **2** (homogenious on Si gel TLC). Structures of **1** and **2** were tentatively proposed (Reference cited) as shown in separate page. Further separation of these compounds and their structure elucidation is in progress

Compounds **3**, **4** and **5** were isolated and charecterised. Compound **3** is β-Sitosterol glucoside, compounds **4** and **5** are iridoid glucosides (Reference cited) and not showed the anticancer activity in this study.

Detailed isolation procedure of chemical constituents from *Wendlandia formosana* was illustrated in Chart-1, data on cytotoxic activity studies of fractions and pure compounds were provided in Table 1 and 2 respectively. NMR data of compounds 1, 2, 3, 4 and 5 were presented in the following pages.

1.2 Studies on active constituents of stem wood of *Wendlandia formosana*

Crude extract [TMCWFW-1, 101% (NUGC 50μg/ml) , 107% (HONE-1 50μg/ml)]; Hexane soluble fraction [TMCWFW-124, 125% (NUGC 50μg/ml), 96%(HONE-1 50μg/ml)]; Ethyl acetate soulble fraction [TMCWFW-125, 111% (NUGC 50μg/ml) 110% (HONE-1 50μg/ml)]; and precipitate from water [TMCWFW-13, 107% (NUGC 50μg/ml) , 104% (HONE-1 50μg/ml)] and water soulble fraction [TMCWFW-126, 105% (NUGC 50μg/ml) , 108% (HONE-1 50μg/ml)] did not show any activity.

(2) Studies on active constituents of root of *Wendlandia formosana.*

To get more quantity of the mixture of active compounds **1** and **2**, investigation of the root of Wendlandia formosana was taken up. The crude methanol extract of root (TMCOB-WFR-1) showed significant activity [TMCOB-WFR-1, HONE-1 46% (50μg/ml), NUGC 25% (50μg/ml)]. Inaddition to the isolation of active compounds **1** and **2**, compounds **6, 7** [WFR-P10(NSTPOD00039)] and **8** [WFR-P8 (NSTPOD00038)] were obtained. Compounds **6** and **7** again a mixture (6mg.) of steroidal derivatives showed **cytotoxic activity** against NUGC 98% (10μM), 30% (50μM), HONE-1 99% (10μM), 81% (50μM). Compound **8** not showed anticancer activity.

Spectroscopic data of compounds **6** and **7** indicated that it is also a mixture of two closely related steroids (homogeneous on TLC). Further separation and structure elucidation is in progress. Structure elucidation of compound **8** is also in progress. Compounds **3**, **4** and **5** which were present in the bark of *Wenlandia formosana* were also present in the root.

Detailed isolation procedure was illustrated in Chart 2 and cytotoxic activity data on pure compounds from the root was also provided in Table 2.

(3) Studies on the active constituents of leaves of *Macaranga tanarius.*

As a part of the program, chemical examination of leaves of *Macaranga tanarius* was taken up.

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During the investigation, thirteen polyphenolics **9** - **21** [NSTPOD00001 – NSTPOD00013] were isolated and characterised. Some polyphenols showed significant cytotoxic activity. Compound **9** [NSTPOD00001; NUGC 88% (10μM), 25% (50μM), HONE-1 90%(10μM), 72% (50μM)], compound **12** [NSTPOD00004; NUGC 103% (10μM), 59% (50μM), HONE-1 102% (10μM), 27% (50μM)], compound **13** [NSTPOD00005; NUGC 55% (10μM), 18% (50μM), HONE-1 29% (10μM), 18% (50μM)], compound **16** [NSTPOD00008; NUGC 96% (10μM), 38% (50μM), HONE-1 99% (10μM), 20% (50μM)], compound **20** [NSTPOD00012; NUGC 82% (10μM), 34% (50μM), HONE-1 94% (10μM), 85% (50μM)], compound **21** [NSTPOD00013; NUGC 100% (10μM), 64% (50μM), HONE-1 92% (10μM), 34% (50μM)],

Activity data of other compounds given in Table 3. Structures of cytotoxic polyphenolics were presented in a separate page. (compounds **9, 12, 13, 16, 20** and **21**)

(4) Synthesis and cytotoxic studies of Cantharidin derivatives.

In addition to the above mentioned work, which was scheduled in the original program, some cytotoxic studies were taken up on the derivatives (**22-59**) of Cantharidin. Cantharidin ,a natural product, was isolated from *Mylabris phalerata* Pallas . Cytotoxic activity data of some cantharidin derivatives was given in the table below. Structures of cytotoxic cantharidin derivatives were shown in separate page. (Compounds **22, 23, 27, 29, 32, 43, 44, 47, 50**) . Isolation procedure of Cantharidin and general scheme of preparation of Cantharidinimides were shown in Chart 3. Cytotoxic activity data of all cantharidin derivatives given in Table 4.

Compd. No.	Compound ID (concentration) 30μM	59T (%)	Hone-1 %)	DLD-1 (%)	SCM-1 (%)	NUGC 10 (50) μM (%)	HONE-1 10 (50) μM (%)
22	TMCOB000009	2.0	2.5	116.0	2.5	99 (2)	100 (3)
23	TMCOB000010	2.5	2.0	50.0	6.5	5 (1)	4 (1)
27	TMCOB000014	1.0	1.0	21.5	1.0		
29	TMCOB000016	5.5	2.0	113.0	12.5	94 (2)	101 (1)
32	TMCOB000019	3.0	1.0	113.5	7.0	96 (2)	108 (1)
43	TMCOB000030	2.5	2.5	109.0	62.0		
44	TMCOB000031	1.0	2.5	112.0	4.0		
47	TMCOB000034	38.0	4.0	109.0	6.5	3 (1)	2 (1)
50	NSTPOD00018					5 (1)	8 (1)

****WFR-P9 (**NSTPOD00014 : 10mg.) (**1** and **2**) (Active compound present in Bark and Root of *Wendlandia Formosana*)

White amorphous powder, ^{1}H NMR (500 MHz, DMSO-d_6): ä **0.66 (3H, s); 0.68 (3H,s)**; 0.79 (3H, d, J=6.4 Hz); 0.83-0.89 (18H, m); **1.02 (3H, s); 1.07 (3H,s)**; 4.28 (br s, 3H), 5.11 (1H, br s); 5.14 (1H, br s).

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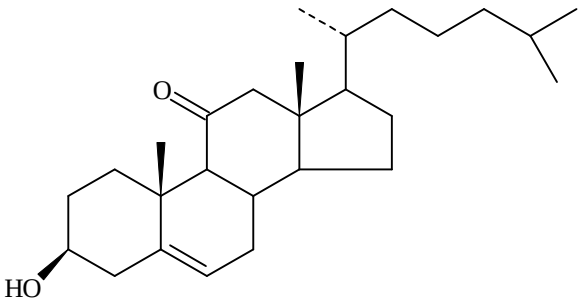
^{13}C NMR (125 MHz, DMSO-d_6): 15.1, 15.2, 16.0, 16.08, 16.86,16.9, 17.0, 18.0, 21.1, 22.0, 22.1, 23.3, 23.4, 23.8, 26.9, 27.5, 28.2, 28.3, 30.2, 30.4, 32.0, 32.1, 32.7, 32.8, 33.0, 36.3, 36.5, 36,6, 38.2, 38.3, 38.4, 38.5, 41.0, 41.3, 41.6, 45.4, 45.7, 46.8, 47.0, 47.1, 52.4, 54.8, 76.8, **121.5, 124.5, 138.2, 143.8, 178.3, 178.6**.



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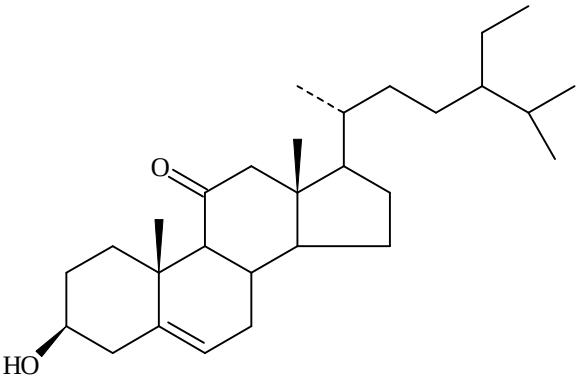

2

The ^{1}H NMR showed characteristic peaks of steroids (reference cited) and the ^{13}C NMR indicates the presence of a carbonyl carbon, an oxygenated carbon and an unsaturation. Carbon spectrum also confirmes that it is mixture of two compounds, duplicate signals are seen for unsaturated and cabonyl and other carbons.

Tentative structures proposed for active compounds mixture, based on NMR data. Isolation of more quantity and obtaining other data is in progress

Ref: G. Notaro, V. Piccialli and D. Sica *J. Nat. Prod.,* **55 ,** 1588-1594, 1992.

WFB-P4 (TMCOB-WFB-1331) (4): Colorless crystals, ^{1}H NMR (500 MHz, D_2O): ä 3.03 (1H, dd, J=3.3 and 7.3 Hz, H-5); 3.17 (1H, t, J=5.1 Hz, H-9); 3.26 (1H, t, J=9Hz, H-2´); 3.29 (3H, s, -OCH_3); 3.33-3.41 (2H, m, H-3´ and H-4´); 3.45 (1H, t, J=9Hz, H-5´); 3.72 (3H, s, -$COOCH_3$); 3.85 (1H, br d, J=10.5 Hz, H-6´); 3.66 (1H, dd, J=5.7Hz and 12.3Hz, H-6´); 4.20 (1H, d, J=15.2 Hz, H-10): 4.28 (1H, d, J=15.2 Hz, H-10); 4.55 (1H, br s, H-6); 4.72 (1H, d, J=8Hz, H-1´); 5.35 (1H, d, J=5.1Hz, 1-H); 5.79 (1H, br s, H-7); 7.46 (1H, s, H-3).

^{13}C NMR (125 MHz, D_2O): ä 40.5 (C-5); 43.8 (C-9); 47.0 (C-11); 50.1 (C-13); 57.4 (C-10); 58.8 (C-6´); 67.5 (C-4´); 70.8 (C-2´); 73.8 (C-5´); 74.5 (C-3´); 78.4 (C-6); 94.4 (C-1´); 107.5 (C-8); 127.0 (C-7); 144.2 (C-4); 151.0 (C-3); 168 (-<u>CO</u>OCH_3).

Ref: Yoshio Takeda, H.Nishimura and H. Inouye, *Phytochemistry* , 1977, Vol **16**, P1300-1301

WFB-12 (TMCOB-1331) (5) : Colorless crystals: ^{1}H NMR (500 MHz,CD_3OD): ä 2.55 (1H, t, 7.8 Hz, H-9); 3.01 (1H, t, 7.4 Hz, H-5); 3.21-3.28 (3H, m, H-2´, 3´, and 4´); 3.38 (1H, t, J= 8.9 Hz, H-5´); 3.61 (1H, dd, J=5.7 and 12.0 Hz, H-6´); 3.73 (3H, s, -$COOCH_3$); 3.84 (1H, br d, J=10.8 Hz, H-6´); 4.20 (1H, d, J=15.6 Hz, H-10); 4.45 (1H, d, J=15.4 Hz, H-10); 4.70 (1H, d, J=8 Hz, H-1´); 4.78 (1H, d, J=2.1 Hz, H-6); 5.0 (1H, d, J=8.9 Hz, H-1); 6.0 (1H, d, J=2 Hz, H-7); 7.6 (1H, d, J=1.3 Hz, H-7).

^{13}C NMR (125 MHz, D_2O): ä 42.5 (C-5); 45.7 (C-6); 51.7 (COO<u>C</u>H_3); 61.6 (C-10); 62.7 (C-6´); 71.5 (C-4´); 74.9 (C-2´); 75.3 (C-6); 77.7 (C-5´); 78.4 (C-3´); 100.4 (C-1´); 101.5 (C-1), 108.2 (C-8); 129.7 (C-7); 151.4 (C-4); 155.3 (C-3); 169.4 (-<u>C</u>$OOCH_3$).

Ref: Yoshio Takeda, H.Nishimura and H. Inouye, *Phytochemistry* , 1977, Vol **16**, P1300-1301

WFB-P3 (TMCOB-WFB-1231) (3): White powder, ^{1}H NMR (500 MHz, DMSO-d_6): ä 0.66 (3H, s, H-18); 0.83-0.86 (9H, m, H-26, 27 and 29); 0.93 (3H, d, J= 6.4 Hz, H-21); 0.99 (3H, s, H-19); 4.88 (1H, m, 3á-H); 4.91 (1H, d, J= 4.7 Hz, H-1´); 5.35 (1H, br s, H-6).

^{13}C NMR (125 MHz, DMSO-d_6): ä 11.6 (C-18); 11.7 (C-29); 18.6 (C-21); 18.9 (C-19); 19.0 (C-26); 19.7 (C-27); 20.5 (C-11); 22.6(C-28); 23.8 (C-15); 25.4 (C-23); 27.7 (C-16); 28.7 (C-25); 29.2 (C-2); 31.3 (C-8); 31.4 (C-7); 33.3 (C-22); 35.4 (C-20); 36.2 (C-10); 36.8 (C-1); 38.9 (C-4); 40.0 (C-12); 41.8 (C-13); 45.1 (C-24); 49.6 (C-9); 55.4 (C-17); 56.1 (C-14); 61.1(C-6´); 70.1 (C-4´); 76.5 (C-2´); 76.7 (C-3); 76.9 (C-3´and 5´); 100.7 (C-1´); 121.2 (C-6) 140.4 (C-5).

WFR-P10 (NSTPOD00039: 6mg, Active) (**6** and **7**): White amorphous powder: ^{1}H NMR (500 MHz, $CDCl_3$): ä 0.73 (3H, s); 0.83-0.86 (12H, m); 0.88-0.91 (12H, m); 0.97 (3H, s); 1.11 (3H, s); 1.18 (3H,s); 1.37 (3H, s); 2.02 (3H,s); 2.03 (3H, s); 2.79 (1H, dd); 4.15 (1H, br s); 4.45-4.51 (2H, m); 5.26 (2H, br s).

^{13}C NMR (125 MHz, DMSO-d_6): ä 15.6, 16.4, 16.6, 16.8, 17.1, 17.5, 18.1, 18.8, 20.2, 21.2, 22.9, 23.3, 23.4, 23.52, 23.56, 23.6, 25.8, 27.4, 27.6, 27.8, 28.0, 28.9, 29.3, 30.6, 31.5, 31.8, 32.4, 32.5, 33.0, 33.2, 33.8, 33.9, 34.4, 36.4, 36.9, 37.6, 37.8, 38.0, 38.2, 39.2, 39.7, 41.0, 41.6, 42.3, 43.0, 44.7, 45.3, 46.5, 47., 51.9, 55.2, 55.3, 64.9, 80.60, 80.9, 91.6, 122.5, 143.5, 170.9, 179.0, 182.5. It is also a mixture of two steroidal derivatives, separation of the mixture and identification of constituents is in progress.

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WFR-P8 (NSTPOD00038) (**8**) : Pale pink amorphous powder, ^{1}H NMR (500 MHz, CD_3OD): ä 2.75 (1H, dd, 2.2, 17.3 Hz); 2.93 (1H, dd, J=4.9, 17.2Hz); 3.33 (1H, s); 4.06 (1H, d, J=3.4 Hz); 4.23 (1H, s); 4.40 (1H, d, J=3.2 Hz); 6.00 (1H, d, J=2.0 Hz); 6.07 (1H, s); 6.09 (1H, s); 6.81 (2H, d, J=8.16); 6.96 (2H, dd, 1.9, 8.2 Hz); 7.01(2H, dd, J=1.8, 8.2); 7.15 (2H, dd, j=1.6, 6.9Hz).

^{13}C NMR (125 MHz): ä 29.2, 29.8, 66.9, 68.0, 81.7, 96.5, 96.6, 98.3, 100.2, 102.4, 104.3, 107.2, 115.6, 115.9, 116.0, 119.8, 120.4, 131.2, 132.4, 145.6, 145.9, 146.3, 146.3, 146.7, 152.1, 152.2, 154.2, 156.5, 156.9, 158.0. Structure elucidation is under investigation.

(5) Conclusion:

1. Five pure compounds (**1**-**5**) were isolated from the stem bark of *Wendlandia formosana*. Compounds **1** and **2** (steroidal ketones) are **cytotoxic** and obtained as close mixture (homogenious on Si gel TLC). Further separation and their structural confirmation is in progress.

2. Stem wood fractions did not show cytotoxic activity.

3. Eight compounds (**1**-**8**) were isolated from the root of *Wendlandia formosana.* Compounds **1** and **2** are **cytotoxic.** Compounds **6** and **7** (steroidal derivatives) showed **cytotoxic** activity. Compounds **6** and **7** were also obtained as a close mixture (homogenious on Si gel TLC). Structure elucidation of compounds **6**, **7** and **8** are in progress and hence their structures were not provided.

4. Thirteen polyphenols (**9**-**21**) were isolated from the leaves of *Macaranga tanarius*. Compounds **9**, **12**, **13**, **16**, **20** and **21** are **cytotoxic.**

5. Thirty eight cantharidin derivatives (**22**-**59**) were synthesised from Cantharidin which was isolated from *Mylabris phalerata*. Compounds **22**, **23**, **27**, **29**, **32**, **43**, **44**, **47** and **50** are **cytotoxic.**

6. Antivirus studies on all compounds and fractions are in progress at NHRI.

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Chart 1

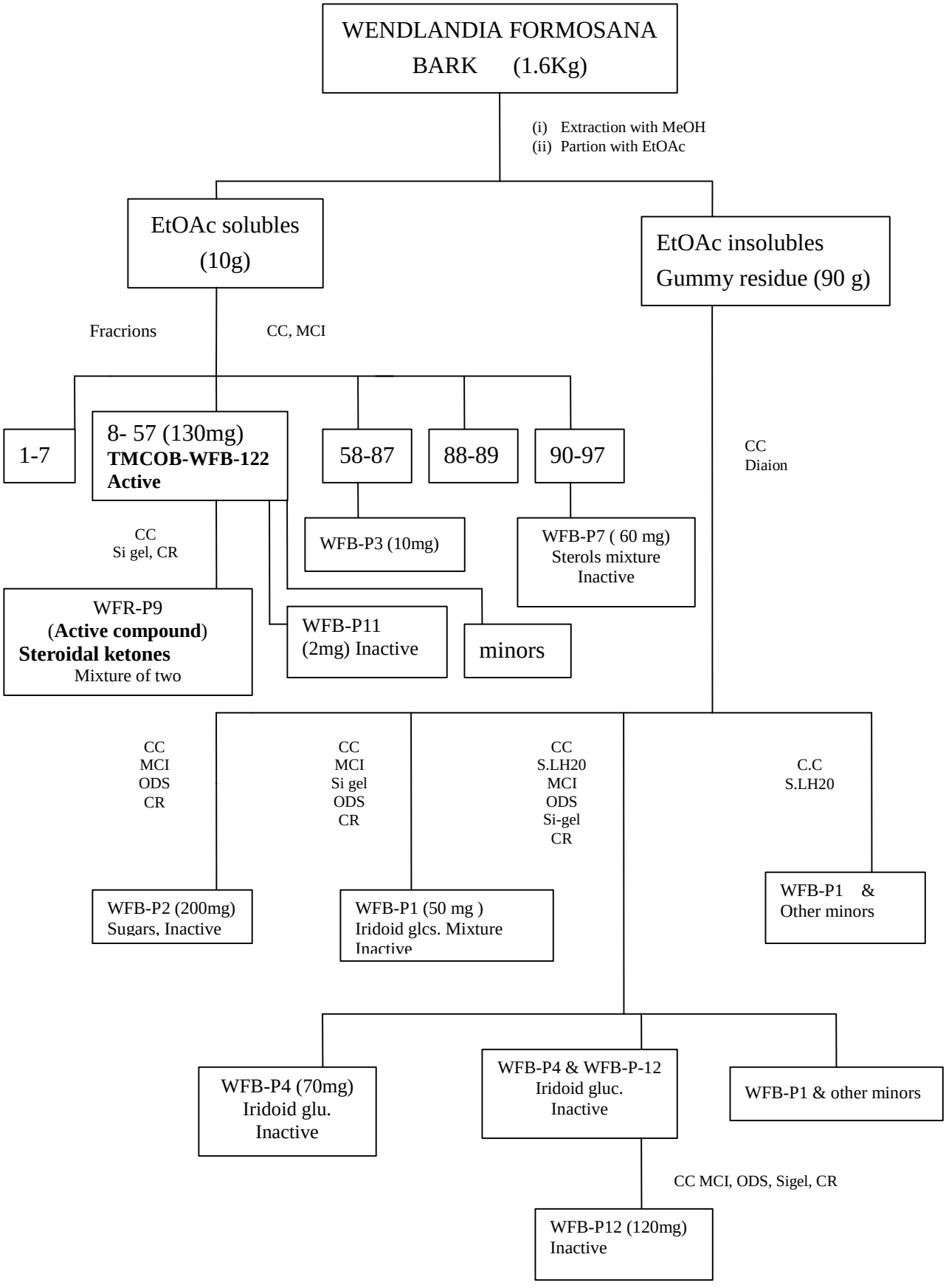

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Table 1. List of fractions from *Wendlandia formosana*

Fraction ID	HONE-1	NUGC
TMCOB-WFB-1231	100%	97%
TMCOB-WFB-1251	91%	98%
TMCOB-WFB-1311	96%	96%
TMCOB-WFB-1321	105%	99%
TMCOB-WFB-1331	98%	93%
TMCOB-WFB-1332	98%	91%
TMCOB-WFB-121	97%	91%
TMCOB-WFB-122 *	**44**%	**19**%
TMCOB-WFB-124	130%	73%
TMCOB-WFB-125	125%	93%
TMCOB-WFB-1**	92%	93%
TMCOB-WFR-1**	**46**%	**25**%
TMCWFB-2-146	78%	**41**%
TMCWFB-2-147	81%	103%

Sample concentration : 50μg/ml

*The active fraction TMCOB-WFB-122 was very small in quantitiy (130 mg) but contains ten compounds (by TLC). So, to isolate active compounds this fraction was compared with EtOAc soluble fraction of the root, the fraction comtaining same compounds was identified(by TLC). To accumulate more quantity of this fraction the bark is being extracted third time.

** TMCOB-WFB-1 and TMCOB-WFR-1 are crude extracts of Bark and Root of *Wendlandia formosana* respectively.

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Table 2 List of compounds from *Wendlandia formosana*

Compound ID	NUGC (10ì M)	NUGC (50ì M)	HONE-1 (10ì M)	HONE-1(50ì M)
NSTPOD00014 (WFR-P9)	98%	**-1%**	61%	**1%**
NSTPOD-00015 (WFB-P11)	101%	97%	99%	106%
NSTPOD00038* (WFR-P8)	95%	88%	94%	99%
NSTPOD00039* (WFR-P10)	94%	**30%**	99%	81%
NSTPOD00040* (WFR-12 Agl.)	97%	70%	101%	103%

NSTPOD-00015 was not active and minor in quantity (3mg), it was not pursuid further
NSTPOD00040 is an aglycon of iridoid glucoside WFB-P12 (TMCOB-WFB-1332)

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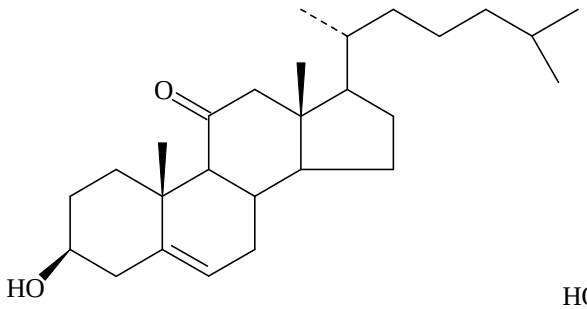
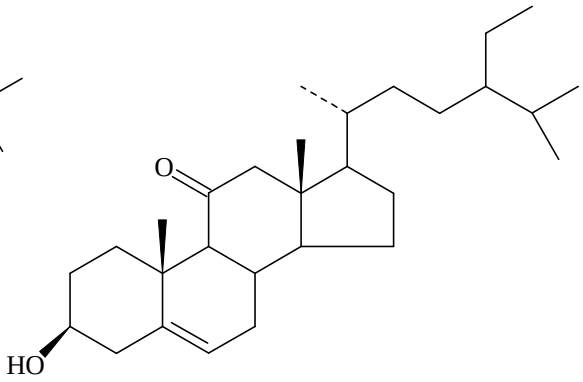
Cytotoxic Steroidal ketones

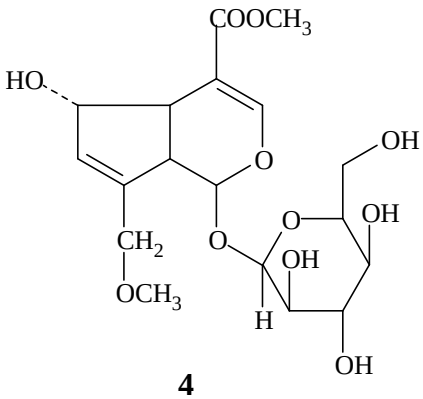


1



2

Iridoid glucosides



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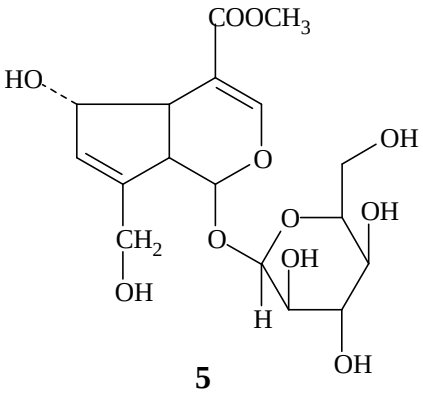
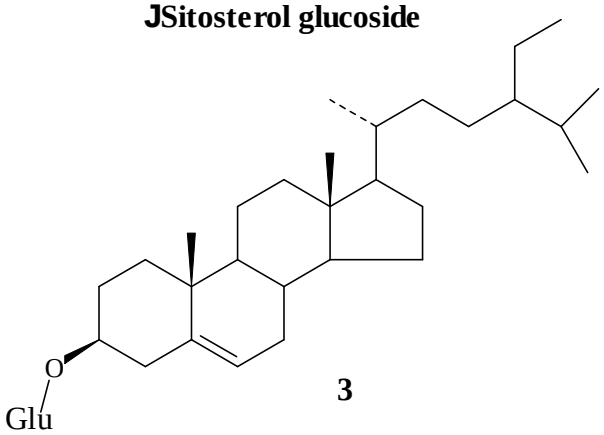


5

JSitosterol glucoside



3

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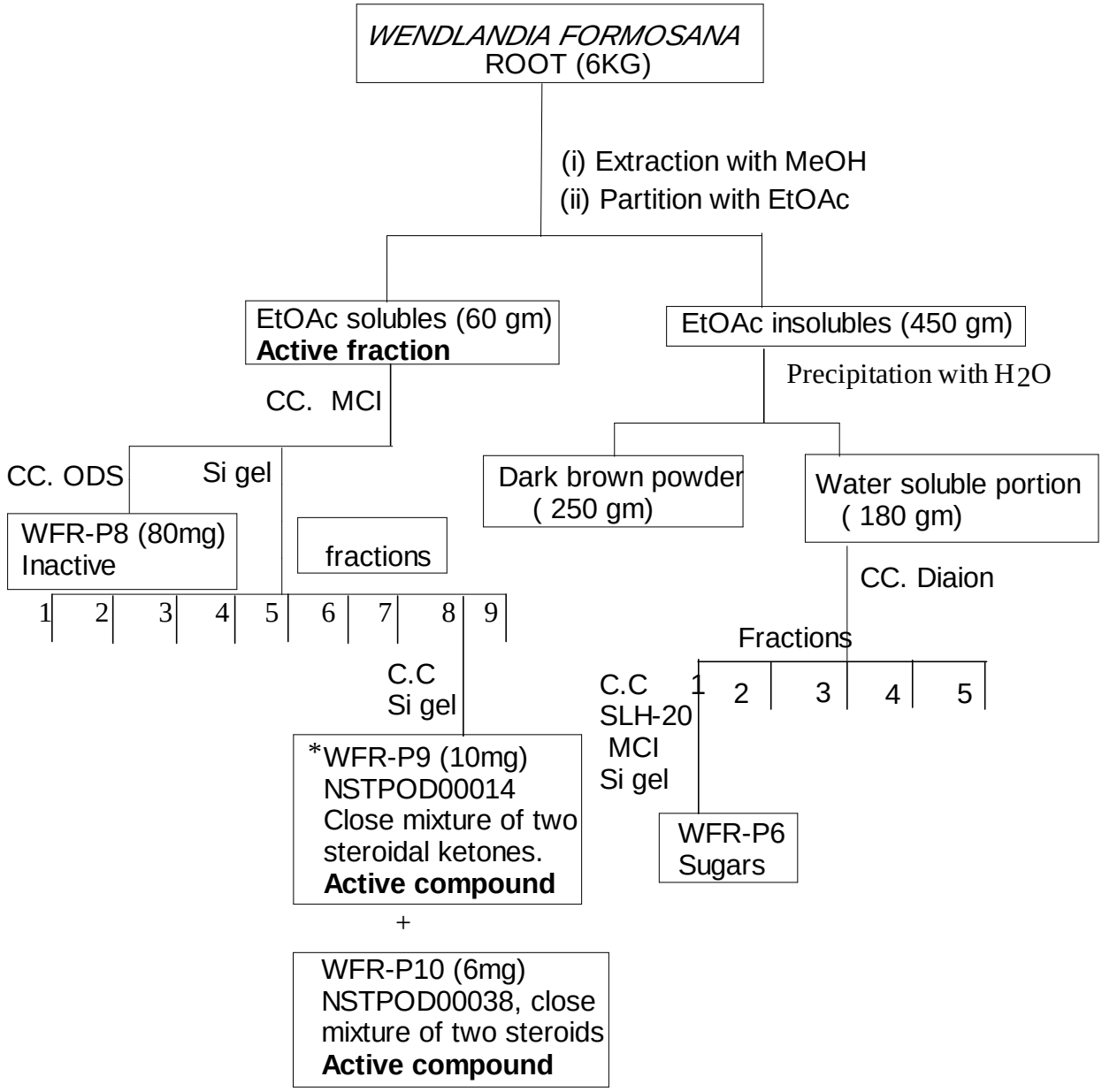
Chart 2



*NSTPOD00014 (WFR-P9) was minor in the active fraction of Bark (TMCOB-WFB-122), so the same compound was isolated from root . In addition to this, NSTPOD00039 was also isolated from the same fraction

*Almost all compounds present in the Bark are present in the root (Checked by TLC)

Chromatography of other fractions is in progress

Iridoid glucosides present in the bark were also present in the root

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Table 3. Cytoxic activity of polyphenols from *Macaranga tanarius*

Compound No.	Compound ID	NUGC (10ì M)	NUGC (50ì M)	HONE-1 (10ì M)	HONE-1 (50ì M)
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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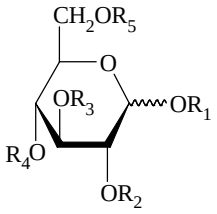
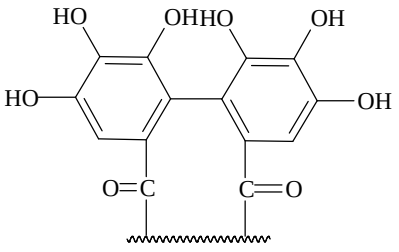
Polyphenolics from the leaves of *Macaranga tanarius*

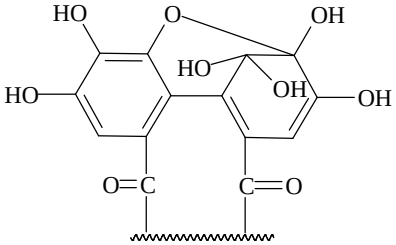


	R1	R2	R3	R4	R5
* **9**	(β)G	H	H	H	H
NM:	(β)G	G	H	H	G
11	(β)G	G	G	H	G
***12**	(β)G	G	H	G	G
***13**	(β)G	G	G	G	G

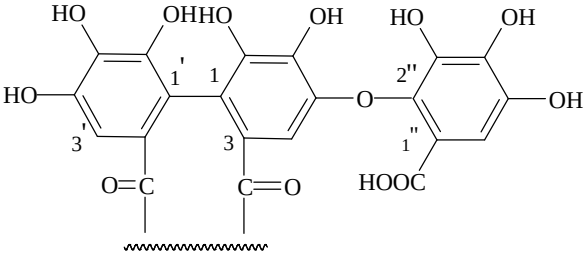
	R1	R2	R4	R3	R5
14	(β)G	H	H	(*R*)HHDP	
15	(β)G	G	G	(*R*)HHDP	
***16**	(β)G	DHHDP	H	H	
17	(β)G	DHHDP	(*R*)HHDP		
18	(β)G	DHHDP	G	G	
19	(β)G	DHHDP	Valoneoyl		
* **20**	(β)G	G	G	(*S*)HHDP	
* **21**	(β)G	G	G	Acyl-A	



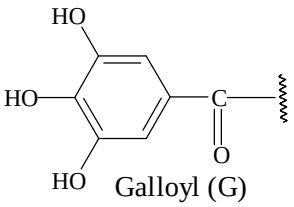
Hexahydroxyldiphenoyl (HHDP)



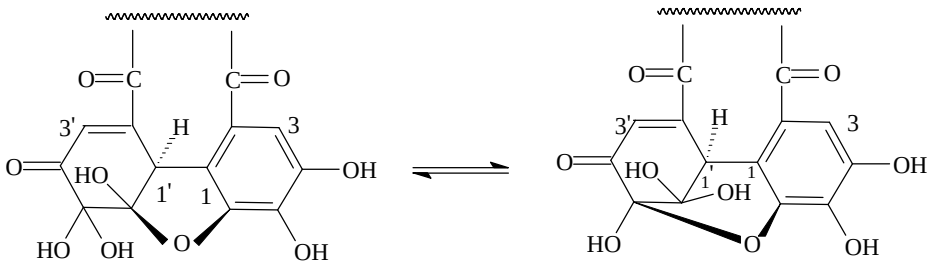
Acyl-A



Valoneoyl



Galloyl (G)



Dehydrohexahydroxydiphenoyl (DHHDP)

*Cytotoxic polyphenols

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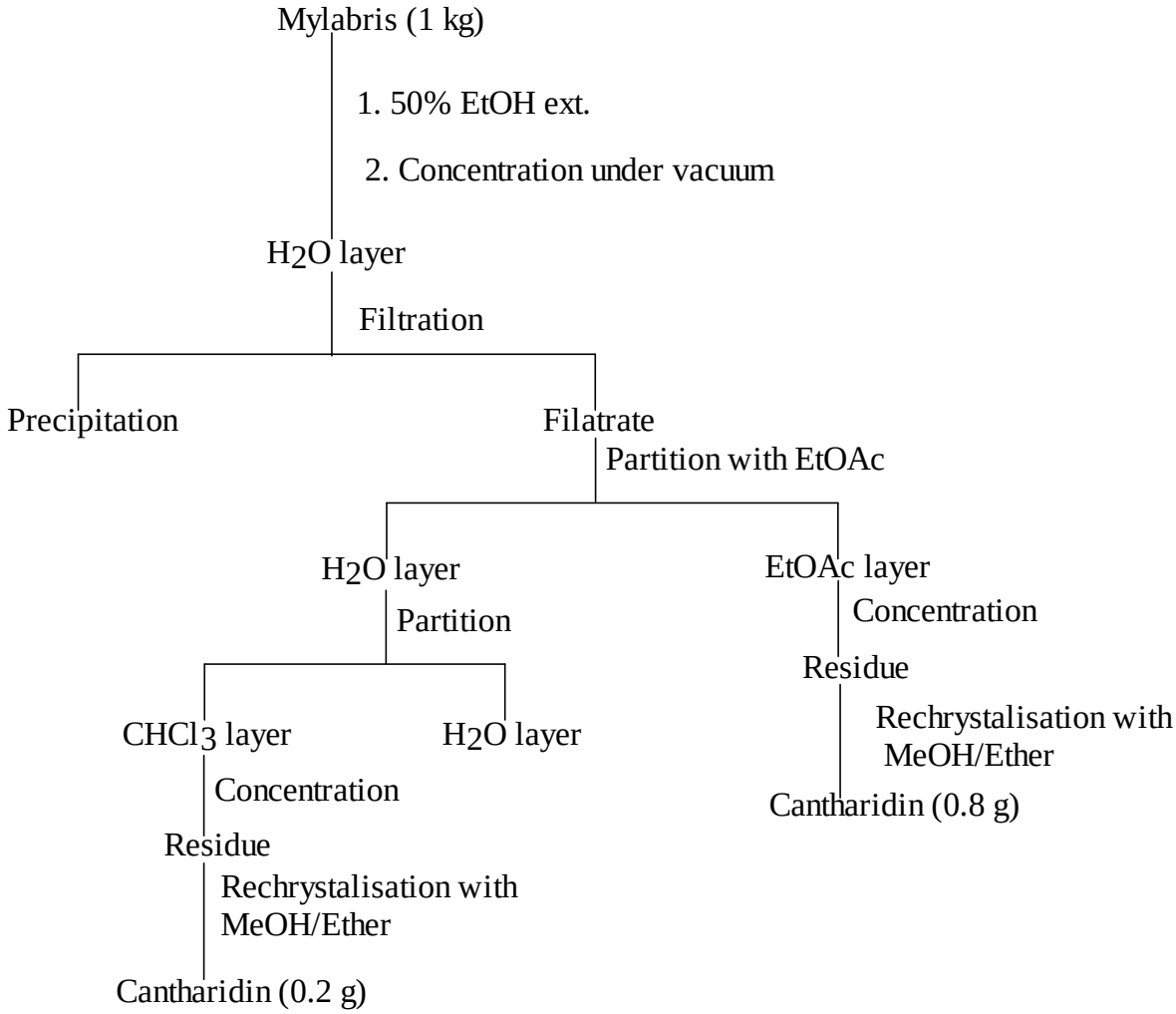
Chart 3 Isolation of Cantharidin from *Mylabris phalerata*



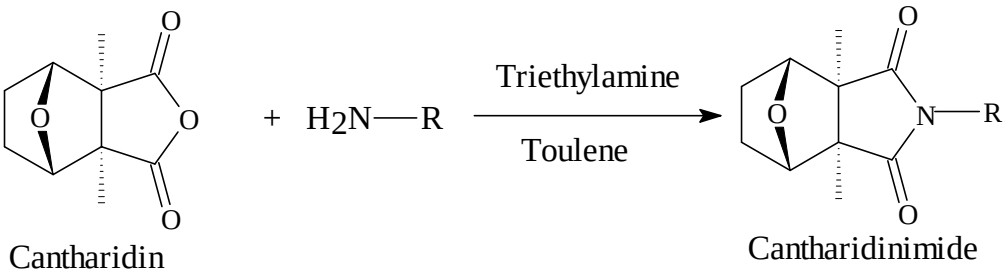


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Table 4. Cytotoxic Activity of Cantharidin derivarives

Compd. No.	Compound ID (Conc. 30μM)	**59T** (%)	**Hone-1** (%)	**DLD-1** (%)	**SCM-1** (%)	**NUGC** 10 (50) μM (%)	**HONE-1** 10 (50) μM (%)
22	**TMCOB00009***	**2.0**	**2.5**	**116.0**	**2.5**	**99 (2)**	**100 (3)**
23	**TMCOB000010***	**2.5**	**2.0**	**50.0**	**6.5**	**5 (1)**	**4 (1)**
24	TMCOB000011	109.5	111.0	101.0	103.5		
25	TMCOB000012	103.5	106.5	105.5	103.5		
26	TMCOB000013	95.5	78.5	104.5	100.5		
27	**TMCOB000014***	**1.0**	**1.0**	**21.5**	**1.0**		
28	TMCOB000015	121.5	116.0	104.0	108.0		
29	**TMCOB000016***	**5.5**	**2.0**	**113.0**	**12.5**	**94 (2)**	**101 (1)**
30	TMCOB000017	119.5	122.0	99.0	111.0		
31	TMCOB000018	96.0	89.0	108.0	105.0	102 (97)	109 (112)
32	**TMCOB000019***	**3.0**	**1.0**	**113.5**	**7.0**	**96 (2)**	**108 (1)**
33	TMCOB000020	119.0	117.0	101.0	107.0		
34	TMCOB000021	121.0	119.0	101.0	107.0		
35	TMCOB000022	116.0	115.5	100.5	104.5		
36	TMCOB000023	117.5	111.5	100.5	103.5		
37	TMCOB000024	114.5	119.0	99.5	102.5		
38	TMCOB000025	112.0	113.5	100.5	101.0		
39	TMCOB000026	123.5	117.5	104.5	107.5		
40	TMCOB000027	113.5	71.5	100.0	106.5		
41	TMCOB000028	122.0	117.0	101.5	108.0		
42	TMCOB000029	109.5	122.5	104.5	106.0		
43	**TMCOB000030***	**2.5**	**2.5**	**109.0**	**62.0**		
44	**TMCOB000031***	**1.0**	**2.5**	**112.0**	**4.0**		
45	TMCOB000032	114.5	113.0	98.5	104.0		
46	TMCOB000033	95.0	107.0	103.5	105.0		
47	**TMCOB000034***	**38.0**	**4.0**	**109.0**	**6.5**	**3 (1)**	**2 (1)**
48	NSTPOD00016					100 (102)	100 (104)
49	NSTPOD00017					107 (102)	110 (114)
50	**NSTPOD00018***					**5 (1)**	**8 (1)**
51	NSTPOD00019					101 (99)	107 (106)
52	NSTPOD00020					102 (101)	106 (105)
53	NSTPOD00021					100 (95)	105 (105)
54	NSTPOD00022					98 (99)	101 (102)
55	NSTPOD00023					96 (100)	98 (105)
56	NSTPOD00024					102 (102)	97 (106)
57	NSTPOD00025					106 (103)	106 (111)
58	NSTPOD00026					104 (102)	109 (108)
59	NSTPOD00027					104 (100)	106 (103)

* Cytotoxic cantharidin derivatives

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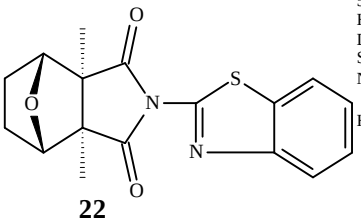
Structures of Cytotoxic Cantharidin derivatives

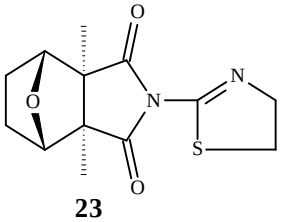
TMCOB000009



59T : 2.0%
Hone-1: 2.5%
DLD-1: 116%
SCM-1: 2.5%
NUGC (10 μ M): 99%
(50 μM): 2%
HONE-1 (10μM): 101%
(50 μM): 1%

22

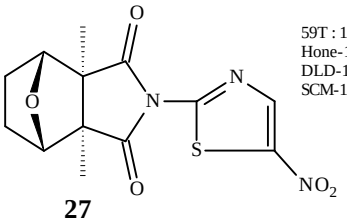
TMCOB000010



59T : 2.5%
Hone-1: 2.0%
DLD-1: 50%
SCM-1: 6.5%
NUGC (10μM): 5%
(50 μM): 1%
HONE-1 (10μM): 4%
(50 μM): 1%

23

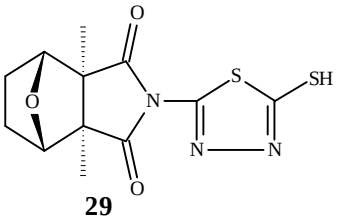
TMCOB000014



59T : 1.0%
Hone-1: 1.0%
DLD-1: 21.5%
SCM-1: 1.0%

27

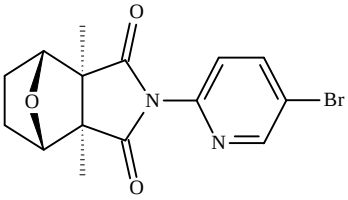
TMCOB000016



59T : 5.5%
Hone-1: 2.0%
DLD-1: 113%
SCM-1: 12.5%
NUGC (10μM): 94%
(50 μM): 2%
HONE-1 (10μM): 101%
(50 μM): 1%

29

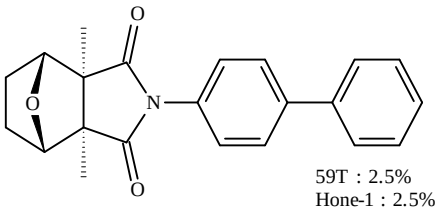
TMCOB000019



59T : 3.0%
Hone-1: 1.0%
DLD-1: 113.5%
SCM-1: 7.0%
NUGC (10μM): 96%
(50 μM): 2%
HONE-1 (10μM): 108%
(50 μM): 1%

32

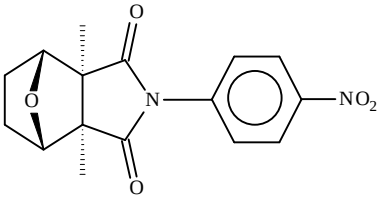
TMCOB000030



59T : 2.5%
Hone-1 : 2.5%
DLD-1 : 109%
SCM-1 : 62.0%

43

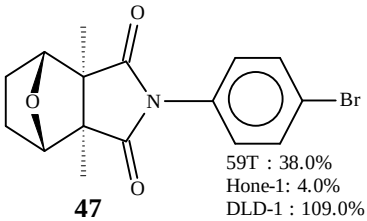
TMCOB000031



59T :1.0 %
Hone-1: 2.5%
DLD-1: 112.0%
SCM-1: 4.0%

44

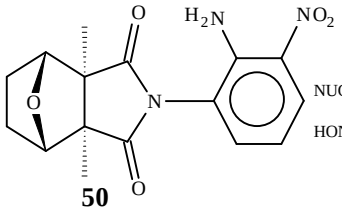
TMCOB000034



59T : 38.0%
Hone-1: 4.0%
DLD-1 : 109.0%
SCM-1 : 6.5%
NUGC (10μM) : 3%
(50 μM) : 1%
HONE-1 (10μM) : 2%
(50 μM) : 1%

47

NSTPOD00018



NUGC (10 μM): 5%
(50 μM): 1%
HONE-1 (10 μM): 8%
(50 μM): 1%

50

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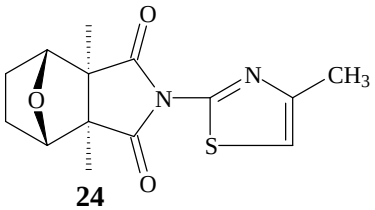
Structures of Inactive Cantharidin derivatives

TMCOB000011



24

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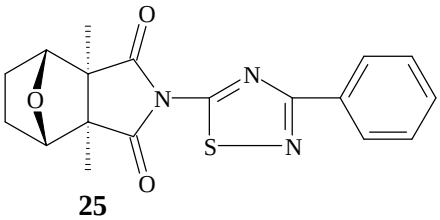
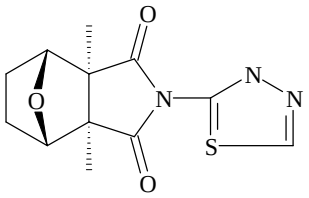


25

TMCOB000013



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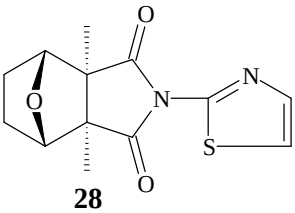

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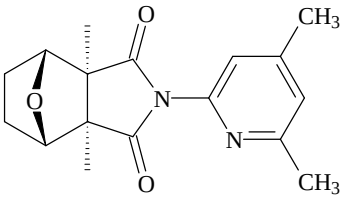

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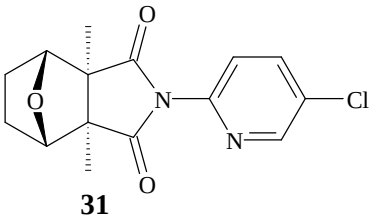

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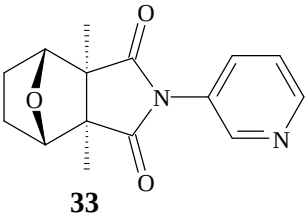

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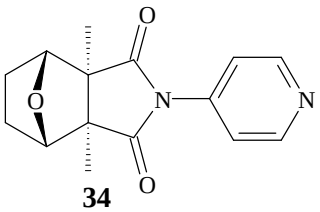

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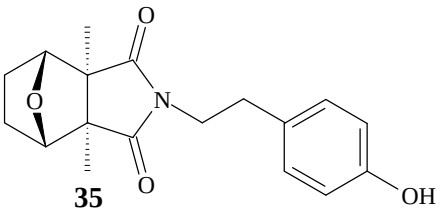

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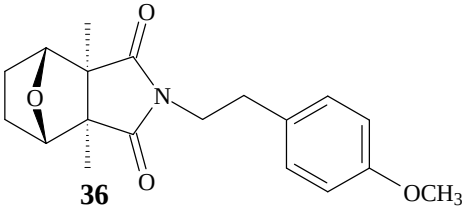

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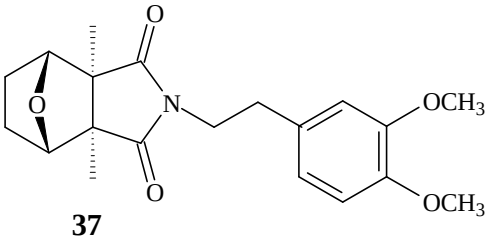

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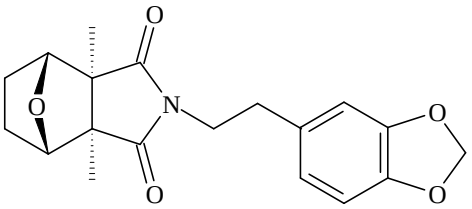

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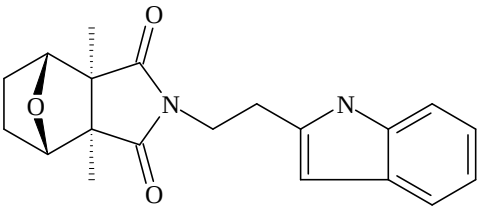

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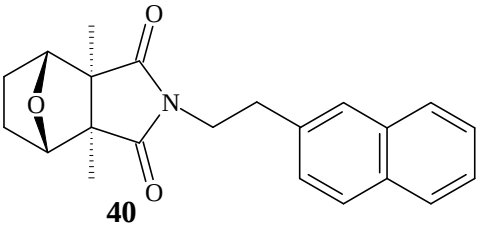

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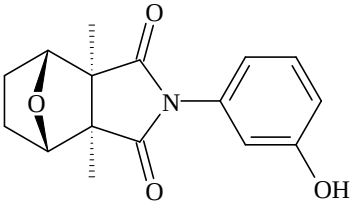

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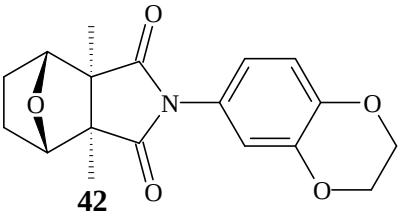

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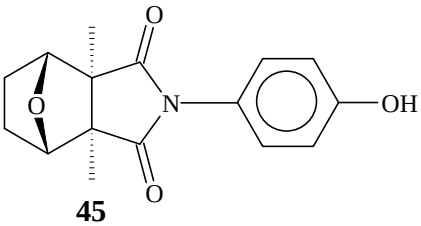

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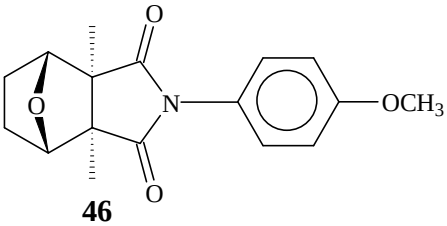

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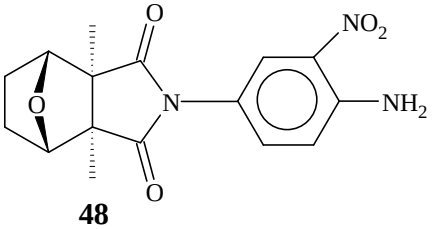

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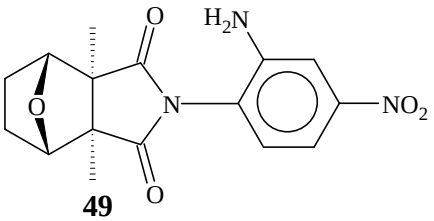

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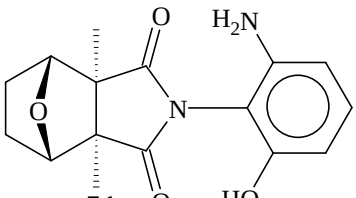

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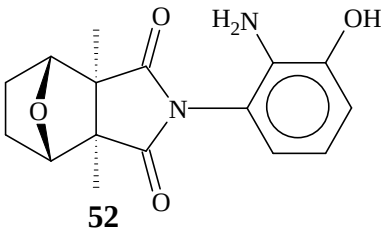

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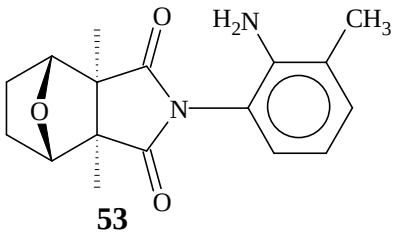

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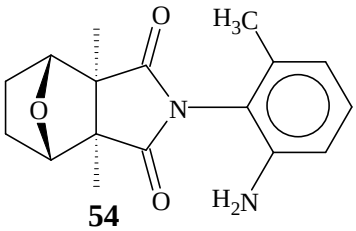

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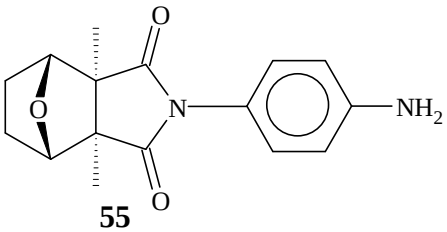

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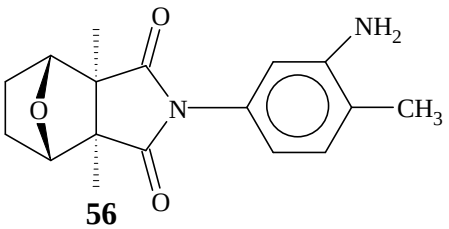

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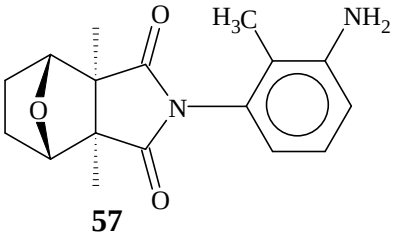

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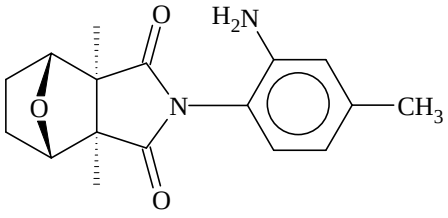

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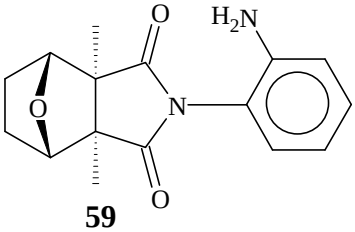

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製藥與生物技術國家型計畫辦公室
National Science and Technology Program in Pharmaceuticals and Biotechnology
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1. 原計畫書之執行進度表。

月次 / 工作項目	第1月	第2月	第3月	第4月	第5月	第6月	第7月	第8月	第9月	第10月	第11月	第12月	備註
1. 水金京，植物材料之採集	**												第一年度 88.12.01~89.12.31
2. 粗萃取及萃取物依極性劃分	**	**											
3. 莖部活性成份之分離、純化		**	***	***	***	***	***	***	***	***	***		
4. 化學結構之探討			***	***	***	***	***	***	***	***	***	**	第 13 月
5. 藥品及器材之採購	***	***	***	***	***	***	***	***	***	***	***	***	
6. 博士後研究員之聘任	***	***	***	***	***	***	***	***	***	***	***	***	***
7. 臨時工僱用	***	***	***	***	***	***	***	***	***	***	***	***	***
8. 篩選用植物萃取之製備	***	***	***	***	***	***	***	***	***	***	***	***	
9. 實驗工作之檢討			**			**			**			*	
10. 研究結果年度整理													**
11. 報告之製作													**
預定進度累計百分比	5	10	15	20	30	40	50	60	70	80	85	90	100 %

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3. **是否達預定進度？如進度落後，請說明原因。**

   本計畫自八十九年一月一日起執行，目前已達預定進度。 同時亦增加研究人力，額外探討 cantharidin **衍生物得到豐富之活性化合物，並開始進行下年度項目之部份工作以求時效。 除了標的植物活性成分探索之工作外，同時進行數十種植物之粗抽取以及** cantharidin **化學修飾以供** NHRI **之活性篩選，其工作繁重，敬請給予人力及經費方面之支持。**

4. **預算執行情形。**

   除了博士後研究員之聘任因人才尋求較難、手續繁雜而略為延遲外，其他預算之執行情形良好。

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研究成果報告附件

第一年已核撥及第二年起申請補助經費

填寫方式請參照原計畫申請書，第二年起經費若有增加部份請另列細項說明。

執行年次 / 補助項目	第一年(89 年 1 月　89 年 12 月)	第二年(90 年 1 月　90 年 12 月)	第三年(91 年 1 月　91 年 12 月)
研究人力費	144,000 (臨時工)	532,800 ※(臨時工，學士專任助理一名)	542,250 ※(臨時工，學士專任助理一名)
研究設備費	0	0	0
赴國外或大陸地區差旅費	0	0	0
出席國際學術會議費用	0	0	0
其他研究有關費用	696,500	750,000	750,000
管理費	50,400	51,000	52,000
小計	890,900	1,333,800	1,344250
國際合作研究計畫差旅費	0	0	0
總計	890,900	1,333,800	1,344250
貴重儀器中心使用額度	300,000	350,000	350,000
博士後研究	共 1 名	共 1 名	共 1 名
博士班研究生獎助金	共　　名	共　　名	共　　名

申請機構或其他單位提供之配合項目			
配合單位名稱	配合補助項目	配合補助金額	配合年次

＊除了標的植物活性成分探索外，同時進行 cantharidin **化學修飾以及數十種植物之粗抽取以供** NHRI **之活性篩選，其工作繁重，敬請給予人力及經費方面之支持。**