國科會專題計畫成果報告 **(NSC Project Reports)**

計畫編號: **NSC88-2113-M-018-004-**

執行期限: **87** 限 **8** 限 **1** 日至 **88** 限 **7** 限 **31** 日

主持人: 人 人 人

執行機構: 國 構 人 構 構 構 構 構

單位名稱: 構 構 稱

稱稱中文摘要

天然物中的類黃酮具有很多醫藥成份，像 Kaempferol 是有潛能抑制人類後天性免疫不全病毒 Type-1 (HIV)整合脢。因爲後天性免疫不全病毒具有把 RNA 反轉錄的能力生成 DNA，生藉由整合脢把這 DNA 嵌入宿主細胞的基因組，也就是促使 HIV-1 能產生複製的行爲，這也是俗稱愛滋病毒感染。最近有文獻報導含有 phenol 結構的化合物，例如 flavones, Caffeic acid phenethyl ester (CAPE), 如 Curcumin 如有抑制 HIV-1 整合脢能力。進稱步知道這類抑制化合物，如具備有二個芳香環，中間有連接者如含有羥基，所以我們的目的將就是想來合成這稱類化合物，進稱步做生化如臨床活性探討，更進稱步研究多羥基的芳香化合物對整合脢的抑制效效。

因爲 Kaempferol 是稱效具有藥性的天然物如它的完整結構，雖然被很多研究群從不同植物中分離出來。但至今仍無文獻報導它的全合成，加上它的結構是由光譜數據所推衍得來。基於上述理由，我們進行它的全合成工作，我們有三項目標(1)提供稱般性方法來合成重要的中間物，(2) 提出最簡潔的合成策略來全合成 Kaempferol，最後(3)利用這個方法來全合成其它類黃酮化合物，如 Quercetin, Morin, Rhamnetin, 如 Fisetin。這其我們提出二效方法來進行 Kaempferol 的全合成: 第稱效是利用 Friedel-Crafts 的方法，第稱效是利用 Cross 醛醇縮合反應進行全合成。

這個 Kaempferol 全合成的策略被提出來，如效成功將會提供稱個最簡潔的方法，而且 Friedel-Crafts 如醛醇縮合反應策略，將會被提供來使用且其它類黃酮化合物的全合成上。

且 且 且: kaempferol, 類黃酮, Quercetin, CAPE, 醛醇縮合

Abstract

The flavone nucleus is part of a large number of natural products and medicinal compounds, which are known as potential human immunodeficiency virus type 1 integrase inhibitor. HIV integrase is an enzyme which incorporates the double-stranded DNA product resulting from the reverse transcription of viral RNA into a host genome, and it shows to be the obligatory for HIV replication. Recently have reported that phenolic moieties in compounds such as flavones, caffeic acid phenethyl ester (CAPE), and curcumin conter inhibitory activity against HIV-1 integrase. In general these inhibitors contain two aryl units, one of which includes the 1,2-dihydroxy pattern, separated by an appropriate linker segment. Therefore, the purpose is to synthesis the end products for studing in HIV integrase assay and in the areas of flavone chemistry and biochemistry. We wish to propose fundamental studies and practical application, specifically a need for the effects of polyhydroxy at the aromatic nucleus.

The Kaempferol is an interesting natural product which play a significant role in the biology activity as well as the structural uniqueness. In addition, although Kaempferol has been isolated from numerous plant sources and its structure proposed on the basis of spectral data, there have been semisynthetic studies. To date, to our knowledge, no kaempferol has been synthesized. Thus, we undertook the total synthesis of kaempferol. The goals of our efforts in this application are to (1) afford a general method for synthesizing key intermediates, (2) present the most concise synthetic strategy for the total synthesis of kaempferol, and (3) utilize this methodology toward the other flavonoids. Two approaches are pursued to determine which strategy for the kaempferal synthesis is viable. One is involved by Friedel-Cratts acylation, and the other is used by aldol condensation.

A new atrategy for the total synthesis of Kaempferol is proposed. The presented convergent synthetic strategy is realized as the most concise synthetic methodology. In addition, the Friedel-Crafts and cross-aldol condensation tactics provide an easy access to the syntheses of other flavonids as well.

Keywords: kampferol, Flavonoids, Quercetin, CAPE, Friedel-Crafts, Aldol.

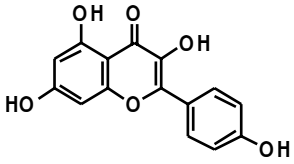
Total synthesis of Kaempferol and other flavonoids (I)

Introduction

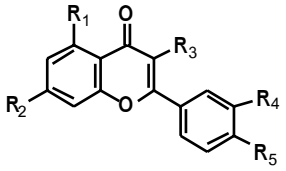
The flavone nucleus is part of a large number of natural products and medicinal compounds.[1] Especially Kaempferol (**1**)[2] and other flavonoids are known as potential human immunodeficiency virus (HIV) type 1 integrase inhibitor.[3] Our previous investigation the flavonoid analogue may prove to be therapeutically useful in analogy to the structural features of CAPE.[4] We extend this assumption that Kaempferol (**1**) or other flavonoids perform inhibitory activity against HIV-1 integrase and tumor. Due to the medicinal imperatives, the scarcity of Kaempferol (**1**) and the clear need for a reliable supply have stimulated us to synthesis these flavonoids. Although Kaempferol (**1**) has been isolated from numerous plant sources and its structure proposed on the basis of spectral data,[5] there have been semisynthetic studies.[6] To date, to our knowledge, no Kaempferol (**1**) has been synthesized. In addition, literature research[7] and our preliminary study show that the flavones have been synthesized from o-hydroxy ketones or chalcone by using the Allan-Robinson[8] or Algar-Flym-Oyamada[9] reaction, but the yields are very low. Herein we report the total synthesis of Kaempferol (**1**) and other flavonoids. We also developed new method for sythesizing neoflavonoids from commercially available cinnamic acid. The total synthesis of Kaempferol (**1**) is described. Highlights of the synthesis include cross aldol condensation and Friedel-Crafts reaction. Several strategies to accomplish this goal are provided.

Result and discussion

The initial proposal was to use ketones and



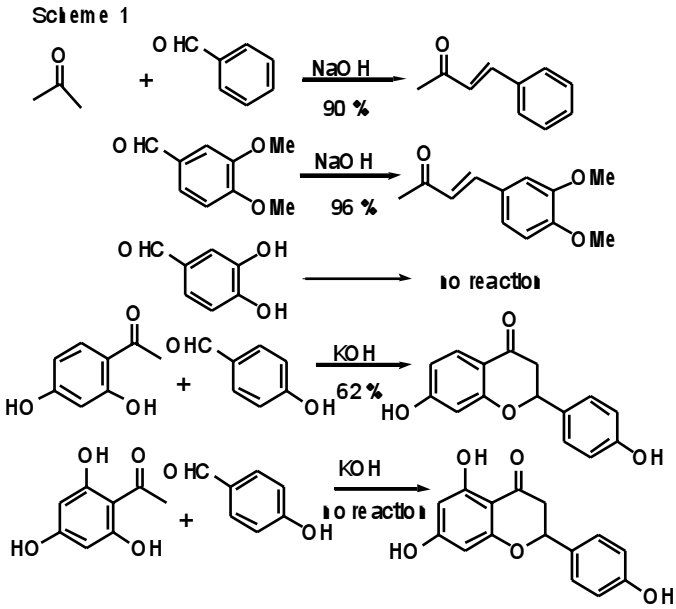
Kaempferol (1)



R1=R2=R3=R4=R5=OH Quercetin (2)
R1=H, R2=R3=R4=R5=OH Fisetin (3)
R1=R2=R3=R5=OH, R4=H Rhamnetin (4)
R1=R2=OMe, R3=H, R4=R5=OH (5)
R1=R2=R4=R5=OMe, R3=H (6)
R1=R2=OH, R3=H, R4=R5=OMe (7)

aldehydes by cross aldol reaction for Kaempferol (**1**) synthesis. (Scheme 1) The scheme 1 showed only starting ketone and side products could be detected/isolated from last aldol reaction under several basis conditions (KOH, NaOH, $NahCO_3$, Na_2CO_3, pyridine, and pyrrolidine). To overcome

Scheme 1

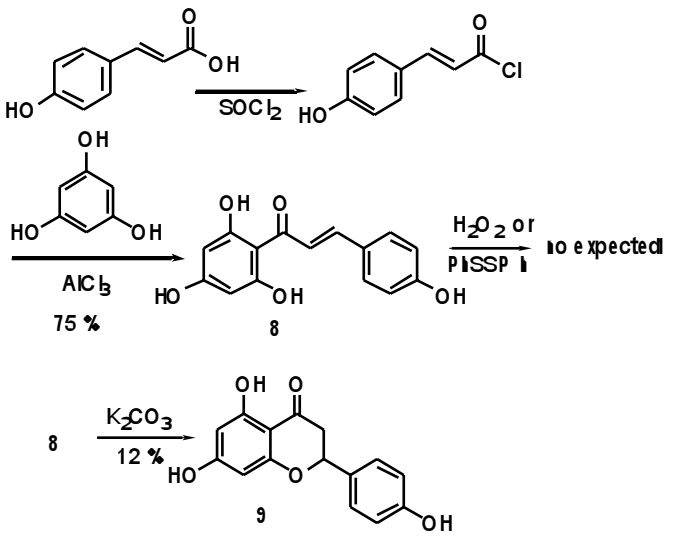


theses technical difficulties, we switch to apply the Friedel-Crafts reaction.

Starting with the commercially available 4-hydroxy cinnamic acid with thionyl chloride,the

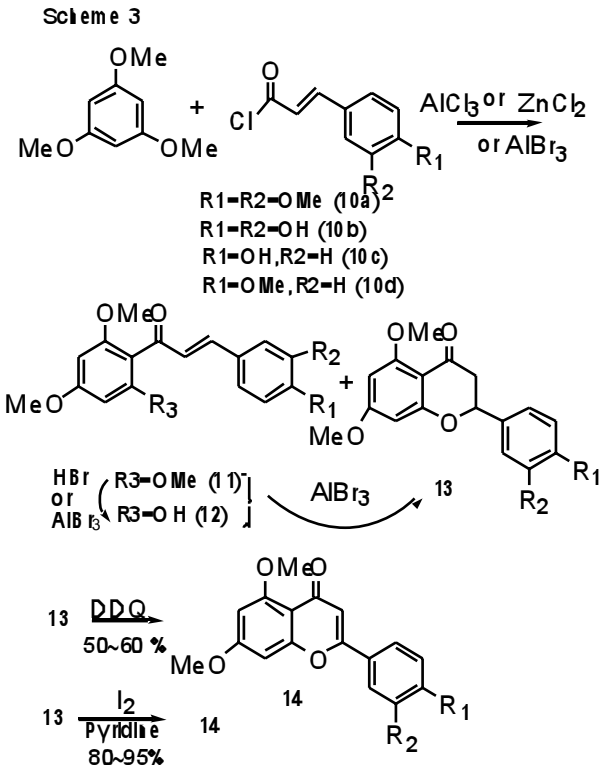
expected acyl chloride can be obtained. The following acylation with phloroglucinol under the Lewis acid catalyst provided tetrahydroxy chalcone. Upon completion of the crucial intermediate, the investigation was focused on the reaction by oxidative cyclization to generate the target (**1**). However, we failed to afford the cyclization product **1** with H_2O_2 or PhSSPh conditions. (Scheme 2) Finally, we can achieve the expected flavonol in the presence of K_2CO_3 at low yield (12%).

Scheme 2



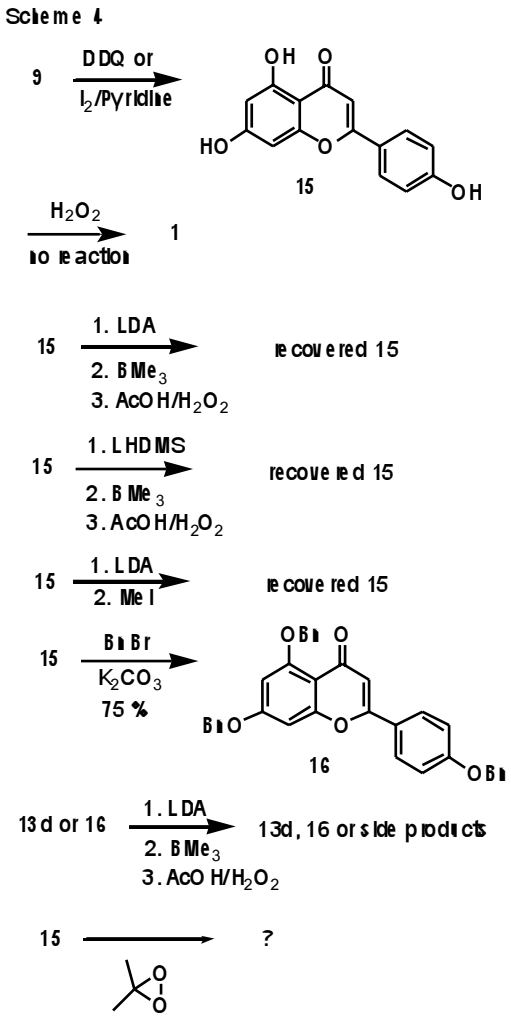
From our observations, the tetrahydroxychalcone made this compound more hydrophilic than other chalcones. With the aim of developing a successful route to **1**, an alternative method was examined (Scheme 3). The hydroxy groups were first protected with methyl group to provide the more lipophilic chalcones. Fortunately, we then sought to employ by Friedel-Crafts with $AlBr_3$ to yield protected flavanol **12**. Subsequent treatment with DDQ or I_2/Pyridine gave the desired product flavanone at high yield. Further attempts to deprotect the methyl groups with BCl_3, BBr_3, $AlCl_3$, BF_3, H_2SO_4, HCl, HBr, HI, Pyridium-HCl, Pyridium-HBr, and $AlBr_3$ were unsuccessful. The chalcones were easily provided by our procedure, we can deprotect the methyl group with Lewis acid, $AlCl_3$ and $AlBr_3$. With the o-hydroxychalcone on hand, it can undergo the cyclization to obtain the protected flavanol. The flavanol was transformed to flavonone with above procedure. (Scheme 4) At this point, the final step of the synthesis was focused on the oxidative reaction of C-3 flavanone. Our results were shown on the scheme 5. However, this proved impossible to afford lithiolation with LDA or LHMDS. The free hydroxy groups of flavaone made this compound more hydrophilic, therefore, it can be converted to an ether compound. Perhaps not surprisingly, only starting materials could be isolated from these reactions.

Scheme 3

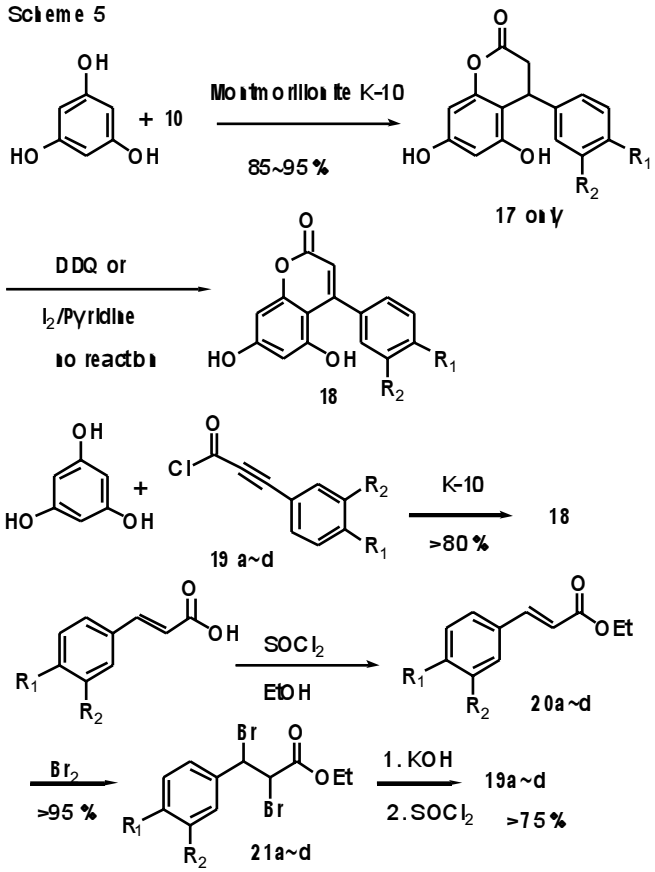


Standard play oxidative-hydroxy reaction of dimethyldioxirane has been performed in the several flavanones. Other project demands, however, didn't allow time for successful oxidative procedure of flavanone. It is worth noting that we have developed a practical synthesis of neoflavonoids catalysed by Montmorillonite K-10. (Scheme 5)

Also, we need reported here the advantage of Pd coupling reaction, we first synthesis of Carpacin's isomer. Finally, Carpacin showed anticarcinogenic potency for exhibiting the effects of GST-inducing activity and inhibition on benzo[a]pyrene induced unscheduled DNA synthesis in rat primary hepatocytes. (Table 1 and 2) Our GST test suggests that further biological assays need underway to evaluate the efficacy of Carpacin as a potential inhibitor of carcinogenesis.



In conclusion, we have synthesized some flavonoids, and a rapid route to neoflavonoids has been achieved. Furthermore, several strategies to accomplish this goal are provided. The oxidative-hydroxy of C-3 flavonone described above serves as a cautionary lesson, which is needed to investigate on the coming project period. Finally, Carpacin was examined as a potential cancer chemopreventive agent.



Experimental Section

Melting points were determined on a Mel Temp II melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Varian MERCURY-400 spectrometer. Chemical shifts are reported in parts per million (δ, ppm) using $CHCl_3$ (δ_H 7.26) as an internal standard. Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were determined on a JEOL JMX-SX/SX 102 A mass spectrometer from National Chung-Hsing University, Taichung. Elemental analyses were performed on a Heracus CHN-OS Rapid spectrometer in the Taichung Instrumentation Center, National Science

Council. Solvents were freshly distilled prior to use from phosphorus pentoxide or CaH_2. THF was from sodium diphenyl ketyl. All reactions were carried out under nitrogen atmosphere otherwise stated. Silica gel (silica gel 60, 230-400 mesh, Merck) was used for chromatography. Organic extracts were dried over anhydrous $MgSO_4$.

Chemicals. Benzo[a]pyrene, collagenase, proteinase K, thiobarbituric acid, thymidine, glycine, [methyl-^{3}H] thymidine, and 1-chloro-2,4-dinitrobenzene were purchased from Sigma Chemical Co., St Louis, MO, USA. Solvents (E. Merck Co., Darmstadt, Germany), aquasol-2 (New England Nuclear, Boston, MA, USA) and medium for cell culture (GIBCO BRL, Grand Island, NY, USA) other reagents or dishes (Nunc, Denmark) were from commercially available sources.

1,2-Methylenedioxy-5-methoxy-4-propynylbenzene (22). A mixture of bromoanisol (200 mg, 0.87 mmol), propynyl magnesium bromide (2.0 mL, 0.5 M, 1.2 equiv), and freshly prepared tetrakis(triphenylphosphine)palladium(0)[7] (50 mg, 5 mol %) in 5 mL of dioxane or THF was placed into a sealable tube equipped with a small magnetic stir bar. The tube was degassed by three cycles of evacuation and refilling with N_2 and sealed under vacuum. The vertical tube was partly immersed in an oil bath heated to 110 °C. The solution was heated under reflux for 48 h. After cooling, the reaction mixture was filtered and the filtrate evaporated. The product was isolated by column chromatography (SiO_2, 1:1 hexane/diethyl ether) to give **22** (164 mg, 100 %) as a yellow solid: mp 40-41 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08 (*s*, 3 H), 3.82 (*s*, 3 H), 5.92 (*s*, 2 H), 6.48 (*s*, 1 H), 6.81 (*s*, 1 H); ^{13}C NMR (40 MHz, $CDCl_3$): δ 4.7, 56.6, 75.7, 88.4, 94.5, 101.4, 104.7, 112.2, 140.7, 148.0, 156.2; Anal. Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30; O, 25.24; Found: C, 69.29; H, 5.55; O, 25.08.

1,2-Methylenedioxy-5-methoxy-4-trans-propenyl benzene (23a). The preparation of **24** was followed by the known procedure.[10] To a solution of **24** (200 mg, 1.1 mmol) in anhydrous THF (20mL) at room temperature was introduced ethylmagnesium bromide (1.7 mL, 1.0 M, 1.5 equiv) by dropewise and the reaction mixture was stirred under a nitrogen atmosphere for 1 h. The clear solution was added 5 N H_2SO_4 (2 mL), and subsequently heated to reflux (50 °C oil bath) for 20 min. Evaporation of the reaction mixture in vacuo gave a light brown solid which was subjected to column chromatography (SiO_2, 1:1 ether/petroleum ether) to give **23a** (175 mg, 82 %) as a white solid: mp 48 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.87 (dt, *J*=6.4, 1.6 Hz, 3 H), 3.77 (*s*, 3 H), 5.90 (*s*, 2 H), 6.00-6.05 (m, 1 H), 6.49 (*s*, 1 H), 6.64 (dd, *J*=16, 1.6 Hz, 1 H), 6.90 (*s*, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.9, 56.7, 94.9, 100.9, 105.3, 119.7, 124.1, 124.9, 141.3, 146.8, 151.2; HRMS (EI) calcd for $C_{11}H_{12}O_3$ (M^+) 192.0786, found 192.0780. The mp and ^{1}H NMR spectra are in agreement with those of natural carpacin provided by Dr. J. Mohandas.[10]

1,2-Methylenedioxy-5-methoxy-4-cis-propenylbenzene (23b). To a shaker suspension of Lindlar catalyst (5 mg of 5 % Pd on $CaCO_3$ poisoned with Pb, Strem) with a drop of quinoline[6] in MeOH (3 mL) was introduced a solution of acetylene **22** (40mg, 0.2 mmol) in MeoH (1 mL) under a 30 psi of hydrogen. The uptake of hydrogen (4.9 mL, 0.2 mmol) was measured quantitatively using a hydrogenation

apparatus, after which the solution was filtered through Celite and evaporated *in vacuo*. Evaporation of the reaction mixture *in vacuo* gave the crude product which was subjected to column chromatography (SiO_2, 1:1 ether/petroleum ether) to give **22b** (39 mg, 97 %) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.83 (dt, *J*=7.2, 1.6 Hz, 3 H), 3.77 (s, 3 H), 5.66-5.83 (m, 1 H), 5.92 (s, 2 H), 6.48 (dd, *J*=11.6, 1.6 Hz, 1 H), 6.54 (s, 1 H), 6.80 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.6, 56.5, 94.7, 101.1, 109.6, 118.6, 124.9, 125.8, 140.5, 146.8, 152.4.

General Procedure for 4-phenylcoumarins from Acyl Chloride with Montmorillonite k-10. The corresponding acid and $SOCl_2$ in nitrobenzene was heated to 70 °C for 2 hrs, the resulting solution was added phloroglucinol and Montmorillonite k-10 for stirring 1 day. The suspension was filtered and the solvent was removed *in vacuo*. The solid was directly subjected to column chromatography (EA:Ether:CH_2Cl_2 1:1:1) to obtain the crude product, which was recrystallized with EA and Hexane to afford the corresponding product at high yield.

Analytical data for selected compounds (unless otherwise indicated, CDCl3 is the NMR solvent). **17b**: ^{1}H NMR [$(CD_3)_2CO$] 2.84 (dd, *J*= 15.7, 2.0 Hz, 1H), 3.05 (dd, *J*= 15.7, 6.6 Hz, 1H), 4.43 (dd, *J*= 6.6, 2.0 Hz, 1H), 6.11 (d, *J*= 2.3 Hz, 1H), 6.25 (d, *J*= 2.3 Hz, 1H), 6.48 (dd, *J*= 8.1, 2.3 Hz, 1H), 6.58 (d, *J*= 2.3 Hz, 1H), 6.69 (d, *J*= 8.1 Hz, 1H), 7.78, 7.83, 8.63 and 8.77 (s, OH, 4H); ^{13}C NMR [$(CD_3)_2CO$] 35.0, 38.6, 96.5, 100.0, 105.7, 115.2, 116.5, 119.4, 135.4, 145.1, 146.3, 154.8, 156.7, 159.2, 168.6; Anal. Calcd for $C_{15}H_{12}O_6$: C, 64.55; H, 5.10. Found: C, 64.33; H, 5.28. **17a**: mp: °C; ^{1}H NMR [$(CD_3)_2CO$+$CDCl_3$] 2.88 (d, *J*= 15.7 Hz, 1H), 3.16 (dd, *J*= 15.7, 5.9 Hz, 1H), 3.62 and 3.70 (s, 6H), 4.43 (d, *J*= 5.9 Hz, 1H), 6.09 and 6.24 (d, *J*= 2.1 Hz, 2H), 6.47 (dd, *J*= 8.2, 1.6 Hz, 1H), 6.82 (d, *J*= 8.2 Hz, 1H), 6.85 (d, *J*= 1.6 Hz, 1H), 9.59 and 9.78 (s, OH, 2H); ^{13}C NMR[$(CD_3)_2CO$+$CDCl_3$] 33.6, 36.9, 55.0, 55.2, 95.6, 99.0, 103.8, 110.1, 111.0, 118.2, 134.1, 147.5, 148.6, 152.7, 154.5, 157.1, 168.2.

References

1. Havsteen, B. *Biochem. Pharmacol.* **1983**, *32*, 1141.
2. (a) Chikao, N.; Nobuyasu, E.; Shinkichi,T.;Akihisa, M.; Koji, K.; Masako, F. *Agric. Biol. Chem.* **1987**, *51*, 139. (b) Joyeux, M.; Lobstein, A.; Anton, R.; Mortier, F. *Planta. Med.* **1995**, *61*, 126.
3. Fesen, M. R.; Kohn, K. W.; Leteutre, F.; Pommier, Y. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 2399.
4. Chen, W. K.; Tsai, C. F.; Liao, P. H.; Kuo, S. C.; Lee, Y. J. *The Chinese Pharm. J.* **1999**, *51*, 271.
5. Sekine, T.; Arita, J.; Yamaguchi, A.; Saito, K.; Okonogi, S.; Morisaki, N.; Iwasaki, S.; Murakoshi, I. *Phytochemistry* **1991**, *30*, 991.
6. Prawat, H.; Mahidol, C.; Ruchirawat, S.; Prawat, U.; Tuntiwachwuttikul, P.; Tooptakong, U.; Talor, W. C.; Pakawatchai, C.; Skelton, B.; White, A. H. *Phytochemistry* **1995**, *40*, 1167.
7. (a) Wagner, H.; Maurer, I.;Farkas, L.; Strelisky, J. *Tetrahedron.* **1977**, *33*, 1411-1414. (b) Nielsen, J. G.; Norgaard, P.; Hjeds, H. *Acta Chem. Scand.* **1970**, *24*, 724-725.
8. Dreyer, D. L.; Tabata, S.; Horowitz, R. M. *Tetrahedron* **1964**, *20*, 2977.
9. Gormley, T. R.; O'Sullivan, W. I. *Tetrahedron* **1973**, *29*, 369.
10. Mohandas, J.; Slaytor, M.; Watson, T. R. *Aust. J. Chem.* **1969**, *22*, 1803.

Table 1. Effect of carpacin on the glutathion S-transferase activity in primary rat hepatocytes.

Treatment	Concentration (mM)	GST activity[a] 12 h (n mole/per min /per mg protein)	GST activity[b] 18 h (n mole/per min /per mg protein)
Normal		286.71 ± 14.36	397.65 ± 7.33
Control (DMSO)	0.2 %	310.93 ± 9.75	409.37 ± 48.61
1a	0.01	332.81 ± 25.36	398.83 ± 58.00
1a	0.02	346.35 ± 15.01*	458.59 ± 7.73**
1a	0.05	408.33 ± 30.78*	515.62 ± 2.54*

a. Primary rat hepatocytes were treated with 1a at various concentrations for 12 h.

b. Primary rat hepatocytes were treated with 1a at various doses for 18 h.
Mean± SD, values are the average of triplicate determinations. **p<0.01 and *p<0.05, compared with control.

Table 2. Effect of carpacin on unscheduled DNA synthesis induced by benzo[a]pyrene in rat primary hepatocytes.

Treatment[a]	cpm/μg DNA[a]	% of inhibition[b]
Control (DMSO)	144 ± 18	–
Benzo[a]pyrene (0.1 mM)	302 ± 9	0
Carpacin (0.01 mM)+Benzo[a]pyrene	300 ± 12	0.6
Carpacin (0.02 mM)+Benzo[a]pyrene	258 ± 9	14.6
Carpacin (0.05 mM)+Benzo[a]pyrene	202 ± 8*	33.1

a. Cell was treated with carpacin and hydroxyurea, then Benzo[a]pyrene (0.1 mM) as described in the experimental section. DNA damage was determined by UDS and expressed as cpm/μg DNA. Mean± SD, values are the average of triplicate determinations.

b. The effect of carpacin on benzo[a]pyrene induced DNA damage was expressed as the fraction of inhibition=[(cpm/μg DNA benzo[a]pyrene treated alone - cpm/μg DNA of carpacin pretreated)/cpm/μg DNA benzo[a]pyrene alone]x100 %

*p<0.05, compared with control.