Academia Journal of Medicinal Plants 9(2): 019-022, February 2021
DOI: 10.15413/ajmp.2020.0143
ISSN: 2315-7720
©2021 Academia Publishing

Academia Publishing

Research Paper

Chemical components isolated from the leaves of *Podocarpus nagi* planted in Fujian and preliminary *in vitro* anticancer activity

Yang Yang[1,2,4,5], Jian-Ping Yong[3*], Olagoke Zacchaeus Olatunde[1,5], Can-Zhong Lu[1,3,4*]

[1]Fujian Institute of Research on the Structure of Matter, Haixi Institute, Chinese Academy of Sciences, Fuzhou, China
[2]Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai, China
[3]Xiamen Institute of Rare-earth Materials, Haixi Institute, Chinese Academy of Sciences, Xiamen, China
[4]ShanghaiTech University, Shanghai, China
[5]University of Chinese Academy of Sciences, Beijing, China

*Corresponding authors. E-mail: jpyong@fjirsm.ac.cn (J.P. Yong), czlu@fjirsm.ac.cn (C.Z. Lu)

Accepted 27th November, 2020

ABSTRACT

The present study was conducted to isolate four compounds (**1-4**) from the ethyl acetate extraction of the *Podocarpus nagi* leaves planted in Fujian. Tri(2,4-di-tert-butylphenyl) phosphate (**4**) were isolated for the first time from this Chinese Traditional Plant Medicine. Other three known compounds were: sugiol (**1**), Podocarpusflavone A (**2**), and II-4',I-7-dimethoxy amentoflavone (**3**). Their structures were elucidated by NMR and XRD analysis. Compound **1** was selected to evaluate the preliminary *in vitro* anticancer activity against cancer cell lines. The results showed that it exhibited higher inhibition against gastric cancer, breast cancer (MCF-7), lung cancer(A549) and Hela cell lines with the inhibitions of 81.48%±1.36, 82.73%±2.08, 53.33%±1.82 and 23.92%±1.2.73, respectively at the concentration of 1.5×10^{-2}M.

Key words: *Podocarpus nagi,* compounds (**1-4**), anticancer activities.

INTRODUCTION

Podocarpus nagi (*P. nagi*, named Zhubai in Chinese) is widely distributed in south districts of Yangtze River, such as Fujian, Hunan, Guangxi and Guangdong, etc. This plant exhibited a wide spectrum of biological activities, such as hemostasis, bone setting, anti-bacterial, anti-tumor, antiviral, antioxidant and detumescence activities (Liao and Wei, 2015). According to the folk records of the Yao Nationality, *P. nagi* has ever been used to treat trauma, stop-bleeding, fractures, knife wounds, gunshot wounds, body odor, eye diseases and colds, etc. The fresh bark or root of *P. nagi* was also used to treat the rheumatoid arthritis (<Zhong Hua Ben Cao>, 1999; Dai, 2009; Yang et al., 2019). Several studies on the chemical components and biological activities of *P.* nagi have been carried out. Ye Yang and Xu Yaming`s groups isolated *P. nagi* lactones from *P. nagi* planted in Guangdong province and evaluated their biological activity. The results showed that most of them exhibited higher antitumor activity (Zheng et al., 2018; Xu and Fang, 1989). Chen Yegao`s group isolated several bioflavonoids and few steroids from the leaves of *P. nagi* grown in Yunnan (Wang et al., 2018). *P. nagi* was also distributed in Nanping of Fujian province, and in recent years, a large scale of *P. nagi* has been planted in Yangli town of Fujian province. In recent years, our research group focused on isolation of chemical components from the leaves and seeds (Yong et al., 2020: 100726). In this study, we isolated and confirmed four compounds from ethyl acetate extract of the leaves of the *P. nagi*: sugiol (**1**), Podocarpusflavone A (**2**), II-4',I-7-dimethoxy amentoflavone (**3**) and tri(2,4-di-tert-butylphenyl) phosphate (**4**) (Figure 1).

Compounds **4** was isolated for the first time from the leaves of *P. nagi*, Compound **1** was selected to evaluate the preliminary *in vitro* anticancer activity against four cancer cell lines using the cell counting kit-8 (CCK-8) method (Tominaga et al., 1999).

EXPERIMENT

General experimental procedures

NMR spectra were recorded on a Bruker AV-400 spectrometer. Column chromatography (CC) was carried

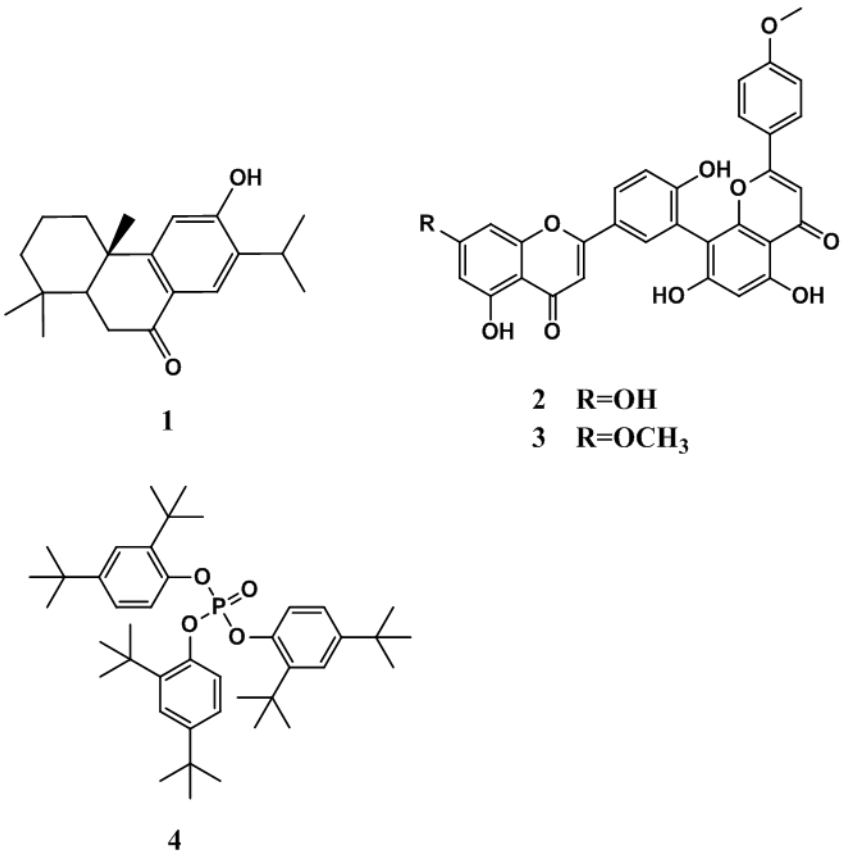


Figure 1: Structures of compounds **1-4**.

out on silica gel (100-200 mesh, Qingdao Marine Chemical Inc., Qingdao, China). Melting points were determined on a XT-4 apparatus equipped with a microscope and are uncorrected. Crystallography data were obtained from Rigaku SuperNova, with CCD detector and X-ray source of Cu Kα radiation (λ = 1.54184 Å). The structure was solved by direct methods with Olex2 Crystallographic Software.

Plant material

The leaves of *P. nagi* were collected in September of 2018 from the Yangli town of Fujian province, China and identified by one of the authors (J.P. Yong).

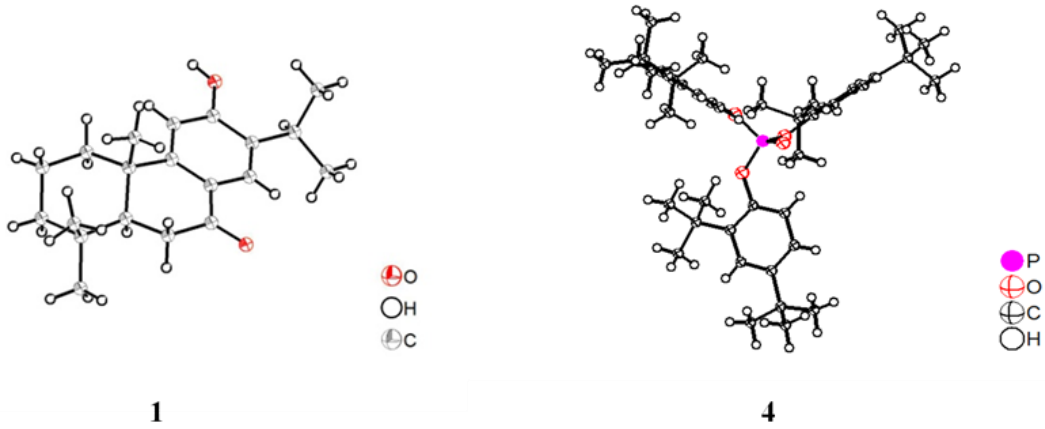
Extraction and isolation

The detailed isolation processes are listed as follows: 10 kg of the air-dried and powdered leaves were added into a 25 L container and the material was dipped in 2 0L 70% ethanol-water solution for one month and then filtered. The filtrate was concentrated under the reduced pressure, the residue was dispersed in 5 L water and extracted with 3×1 L ethyl acetate. The ethyl acetate layers were combined and concentrated under the reduced pressure to obtain another residue, which was rechromatographed over a column of silica gel with petroleum ether, petroleum ether–ethyl acetate ($V_{\text{petroleum ether}}$:$V_{\text{ethyl acetate}}$, 10:1 to 0:1) as eluents to obtain 150 different polar fractions (Fr 1 to Fr 150). After HPLC analysis, we selected 10 fractions and combined for further isolation to obtain another 6 fractions: fraction **1**(petroleum ether as eluent); fraction **2**($V_{\text{petroleum ether}}$:$V_{\text{ethyl acetate}}$, 10:1 as eluent); fraction **3**($V_{\text{petroleum ether}}$:$V_{\text{ethyl acetate}}$, 5:1 as eluent); fraction **4**($V_{\text{petroleum ether}}$:$V_{\text{ethyl acetate}}$, 2:1 as eluent); fraction **5**($V_{\text{petroleum ether}}$:$V_{\text{ethyl acetate}}$, 1:1 as eluent); fraction **6**(ethyl acetate as eluent).

Compound **1**(a sesquiterpene) isolated from fraction **3**; compounds **2** and **3**(bioflavonoids) isolated from fraction **5**; compound **4**(a phosphate) isolated from the fraction **4**. Their structures were elucidated by NMR (^{1}H-NMR and ^{13}C-NMR) and compounds **1** and **4** were also confirmed using XRD analysis (Figure 2). Full spectroscopic data for compounds **1-4** can be found in the Supporting information.

Preliminary *in vitro* anticancer evaluation

Compound **1** was selected to evaluate for the preliminary *in*



1 **4**

Figure 2: Crystal structures of compounds **1** and **4**.

vitro anticancer activity against gastric cancer, breast cancer (MCF-7), lung cancer(A549) and Hela cell lines using the CCK-8 method. Briefly, the cancer cell lines were seeded in 96-well plates (5000 cells/well) with 100 µL DMEM supplemented with 10% fetal bovine serum, and cultured at 37°C in a humidified CO_2 incubator (95% air, 5% CO_2) for 24 h. While the cell lines grew to 90% in logarithmic growth, the culture medium was removed from each well, and 100 µL fresh DEME was added to each well. Then, 10 µL solutions of compound **1** was added into each well (every compound was repeated for 5 times) and the plates were incubated for another 48 h at 37°C. Subsequently, 10 µL CCK8 was added to each well, and the plates were cultured at 37°C for another 4 h. The optical density was measured at a wave-length of 450 nm on an ELISA microplate reader. DMEM and DMSO solution (V/V: 10/1) was used as a negative control. The results were expressed as the inhibition calculated at the ratio [(1-(OD_{450} treated/ OD_{450} negative control)) ×100].

RESULTS AND DISCUSSION

In this study, we isolated four compounds from the leaves of *P. nagi*, and they were characterized using NMR, XRD and HR-MS.

Compound **1**: $C_{20}H_{28}O_2$, colorless columnar crystal, m.p.: 246-248°C; HR-MS for $C_{20}H_{28}O_2Na$, $[M+Na]^+$: Cal. 323.1982, found: 323.1982. Main crystallographic data: monoclinic, space group $P2_1$; a =9.54, b =14.17, c =12.70Å, α=90°, β=90°, γ=90°, V =1716.81 $Å^3$, Z = 4, d = 1.162 g/cm^3. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm) data as follows: 0.88(3H, s, H-18), 0.94(3H, s, H-19), 1.12(3H, s, H-20), 1.16(6H, d, J= 8.0Hz, H-16, 17), 1.26(dd, J=4.0, 4.0Hz, H-6), 1.43(dd, J=4.0,4.0Hz, H-1),1.46(d, J=4.0Hz, H-3), 1.61(dd, J=4.0, 4.0Hz, H-15),1.72(d, J=4.0Hz, H-1), 1.76(d, J=4.0Hz, H-3), 2.16(d, J=12 Hz, H-6), 2.46(dd, J=4.0, 4.0Hz, H-2), 2.50(d, J=4.0Hz, H-2), 6.78(1H, s, H-11), 7.65(1H, s, H-14), 10.25(1H, s, OH-12). HPLC showed that the purity of compound **1** was 97.33%, chromatography condition: $V_{methanol}$: V_{water}=7:3, detection wavelength: 254 nm. The single-crystal data analysis results were ideal. By the comparison of the compounds physical data with those previously reported (Zhao et al., 2016), compound **1** was identified as sugiol.

Compound **2** was yellow amorphous powder obtained from the fraction **5**, m.p.:234-236°C; HR-MS for $C_{31}H_{20}O_{10}Na$, $[M+Na]^+$: Cal. 575.0949, found: 575.0949. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm) and ^{13}C-NMR(100 MHz, DMSO-d_6, δ, ppm) data as below: 3.37(3H, s, H-II-4'), 6.15(1H, d, J=2.4Hz, H-I-6'), 6.36(1H, s, H-II-6), 6.36(1H, d, J=4.0Hz, H-I-2'), 6.67(1H, s, H-I-6), 6.69(1H, s, H-I-8), 6.76(1H, s, H-I-2), 6.80(1H, s, H-II-2), 7.10(1H, d, J=8.0Hz, H-I-3'), 7.52(1H, s, H-II-2'), 7.54(1H, s, H-II-6'), 7.96(1H,d, J=2.0Hz, H-II-2'), 7.97(1H,d, J=2.4Hz, H-II-3'), 10.26(1H, s, OH-I-7), 10.57(1H, br s, OH-I-4'),10.81(1H, s, OH-II-7),12.93(1H, s, OH-I-5), 13.06(1H, s, OH-II-5'); 56.56(OCH_3-II-4'), 94.56(C-I-8), 99.12(C-I-6), 99.37(C-II-6), 100.00(C-I-4), 103.10(C-II-4), 103.47(C-II-8), 104.13(C-I-2), 104.21(C-I-3'), 104.48(C-II-3'), 116.28(C-II-5'), 116.68(C-I-5'), 120.48(C-II-1'), 121.45(C-I-5), 121.91(C-II-2'), 128.45(C-II-6'), 131.93(C-I-6'), 154.99(C-II-9'), 157.89(C-I-9), 160.07(C-I-4'), 160.07(C-II-7), 161.04(C-I-1), 161.55(C-I-7), 161.95(C-I-1'), 162.41(C-II-1), 164.21(C-II-2), 164.32(C-II-3), 164.64(C-II-5), 170.69(C-II-9),182.26(C-I-3), 182.65(C-II-3). HPLC showed that the purity of compound **2** was 96.70%, chromatography condition: $V_{methanol}$: V_{water}=7:3, detection wavelength: 254 nm. By comparison of the compounds spectrum data with those previously reported (Qiao et al., 2019), compound **2** was identified as Podocarpusflavone A.

Compound **3** was yellow amorphous powder obtained from the fraction **5**, m.p.:280-282°C; HR-MS for $C_{32}H_{22}O_{10}Na$, $[M+Na]^+$: Cal. 589.1105, found: 589.1105. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm) and ^{13}C-NMR(100 MHz, DMSO-d_6, δ, ppm) data as below: 3.76(3H, s, H-I-4'), 3.84 (3H, s, H-II-4'), 6.20(1H, d, J=2.0Hz, H-I-6'), 6.42(1H, s, H-II-6), 6.48(1H, d, J=4.0Hz, H-I-2'), 6.91(1H, s, H-I-6),

6.93(1H, d, *J*=2.8Hz, H-I-3'), 6.95(1H, s, H-I-8), 7.37(1H, d, *J*=8.0Hz, H-II-2'), 7.61(1H, s, H-I-2), 7.63(1H, s, H-II-2), 8.06(1H, d, *J*=2.4Hz, H-II-6'), 8.21(2H, dd, *J*=4.0, 4.0Hz, H-II-3', H-II-5'), 10.85(2H, s, OH-I-7, OH-II-7), 12.93(1H, s, OH-I-5), 13.07(1H, s, OH-II-5'),; 55.52(OCH_3-I-4'), 55.92(OCH_3C-II-4'), 94.13(C-I-8), 98.65(C-I-6), 98.90(C-II-6), 103.24(C-I-4), 103.67(C-II-4), 103.68(C-II-8), 103.80(C-I-2), 111.74(C-I-3'), 114.56(C-II-3'), 114.85(C-II-5'), 121.58(C-I-5'), 122.54(C-II-1'), 122.84(C-I-1'), 127.83(C-II-2'), 128.26(C-II-6'), 130.90(C-I-6'), 154.36(C-II-9'), 157.42(C-I-9), 160.44(C-I-4'), 160.63(C-I-5), 160.73(C-II-7), 161.45(C-I-1), 161.49(C-I-7), 161.79(C-I-1'), 162.23(C-II-1), 162.90(C-II-2), 163.12(C-II-3), 163.35(C-II-5), 164.21(C-II-9), 181.80(C-I-3), 182.12(C-II-3). HPLC showed that the purity of compound **3** was 99.25%, chromatography condition: $V_{methanol}$: V_{water}=7:3, detection wavelength: 254 nm. By comparison of the compounds spectrum data with those previously reported (Suarez et al., 2003), compound **3** was identified as II-4',I-7-dimethoxy amentoflavone.

Compound **4**: $C_{42}H_{63}O_4P$, white colorless single-crystal, m.p.: 600-601°C. Crystallographicdata: monoclinic, space group $P2_1$; a=15.48, b=16.13, c=15.92Å, α=90°, β=90.96°, γ=90°, V=3972.7Å^3, Z=111, d=1.108 g/cm^3. The single-crystal data analysis results were ideal. By comparison of the compounds physical data with those previously reported (Vinuchakkaravarthy et al., 2010), compound **4** was identified as tris(2,4-di-tert-butylphenyl) phosphate.

The preliminary *in vitro* anticancer evaluation showed that compound **1** exhibited higher inhibition against gastric cancer, breast cancer (MCF-7), lung cancer(A549) and Hela cell lines with the inhibitions of 81.48%±1.36, 82.73%±2.08, 53.33%±1.82 and 23.92%±2.73, respectively at the concentration of 1.5×10^{-2}M, which can explain the reasons why the leaves of *P. nagi.* exhibited higher anticancer activity in the previously published articles.

CONCLUSION

In this study, four compounds were isolated and confirmed from the leaves of *P. nagi.* During isolation of compounds 1, 2 and 3, we used the PTLC together with recrystallization methods. Compound **1** exhibited higher anticancer activity. Based on this result, study on more new compounds together their biological evaluation will be carried out soon.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (21875252) and Self-created Area Project of Major Science Technology Innovation Platform of Xiamen (3502ZCQ20171002).

REFERENCES

Dai B (2009). Chinese modern Yao medicine. Guangxi science and Technology Publishers, Guangxi. pp. 257-259

Liao ZY, Wei W (2015). Studies on volatile constituents and their anti-tumor activitiesfrom the peel and shell of *Podocarpus nagi* fruits. Her. Med. 34: 609-612.

Qiao YL, Liu XL, Li X, *et al* (2019). Biflavonoids from *Juniperus oblonga* inhibit organic anion transporter 3. Biochem. Biophys. Res. Commun. 509(4): 931-936.

State Administration of Traditional Chinese Medicine Editorial Board of <Zhong Hua Ben Cao > (1999). Zhong Hua Ben Cao. Shanghai Scientific & Technical Publishers, Shanghai. pp. 813-817

Suarez AI, Diaz MB, Delle MF, Compagnone RS (2003). Biflavonoids from *Podocalyx loranthoides*. Fitoterapia. 74(5): 473-475.

Tominaga H, Ishiyama M, Ohseto F, Sasamoto K, Hamamoto T, Suzuki K, Watanabe M (1999). A water-soluble tetrazolium salt useful for colorimetric cell viability assay. Anal. Commun. 36(2): 47-50.

Vinuchakkaravarthy T, Sangeetha CK, Velmurugan D (2010). Tris(2,4-di-tert-butylphenyl) phosphate. Acta Crystallogr. Sect. E Struct. Rep. Online. 66(Pt 9): o2207-8

Wang Q, Yang Z, Peng W, Zhang YK, Chen Y (2018). Study on the Constituents of *Podocarpus nagi*. J. Hainan Normal Univ. (Nat. Sci.). 31(1): 1-5.

Xu YM, Fang SD (1989). Chemical Composition of Podocarpaceae I. Anti-Tumor Chemical Composition in *Podocarpus nagi*. Acta Chimi. Sin. 47(11): 1080-1086.

Yang Y, Yong JP, Lu CZ (2019). Chemical and biological progress of *Podocarpus nagi*. Biomed. Res. Rev. 2(3): 1-5.

Yong JP, Lu CZ, Zhang SB (2020). Preparation method and use of *Podocarpus nagi* kernel oil. New Australian Innovation Patent, Application Number: 2020: 100726.

Zhao SM, Chou GX, Yang QS, Wang W, Zhou JL (2016). Abietane diterpenoids from *Caryopteris incana* (Thunb.) Miq. Org. Biomol. Chem. 14(14): 3510-3520.

Zheng YD, Bai G, Tang CP, Ke CQ, Yao S, Tong LJ, Feng F, Li Y, Ding J, Xie H, Ye Y (2018). 7α,8α-Epoxynagilactones and their glucosides from the twigs of *Podocarpus nagi*: Isolation, structures, and cytotoxic activities. Fitoterapia. 125: 17-183.

Cite this article as:

Yang Y, Yong JP, Olatunde OZ, Lu CZ (2021). Chemical components isolated from the leaves of *Podocarpus nagi* planted in Fujian and preliminary *in vitro* anticancer activity. Acad. J. Med. Plants. 9(2): 019-022.

Submit your manuscript at:
https://www.academiapublishing.org/journals/ajmp/

SUPPORTING MATERIALS

1. Crystllographic data and structure refinement

(1) Sugiol:

Table 1: Crystal data and structure refinement for Sugiol **(1).**

Identification code	Sugiol
Empirical formula	$C_{20}H_{28}O_2$
Formula weight	300.21
Temperature/K	100.0(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	9.5425(3)
b/Å	14.1702(6)
c/Å	12.6965(4)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	1716.81(11)
Z	4
ρ_{calc}g/cm^3	1.162
μ/mm^{-1}	0.565
F(000)	656.0
Crystal size/mm^3	0.2 × 0.1 × 0.05
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	9.352 to 138.64
Index ranges	-11 ≤ h ≤ 11, -17 ≤ k ≤ 16, -15 ≤ l ≤ 11
Reflections collected	5429
Independent reflections	2973 [R_{int} = 0.0541, R_{sigma} = 0.0824]
Data/restraints/parameters	2973/0/205
Goodness-of-fit on F^2	1.207
Final R indexes [I>=2σ (I)]	R_1 = 0.0624, wR_2 = 0.1798
Final R indexes [all data]	R_1 = 0.0813, wR_2 = 0.1876
Largest diff. peak/hole / e Å^{-3}	0.29/-0.26
Flack parameter	0.1(4)

Table 2: Bond Lengths for Sugiol **(1).**

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O001	C007	1.238(6)	C009	C00H	1.526(8)
O002	C008	1.363(6)	C00A	C00C	1.525(7)
C003	C006	1.396(7)	C00B	C00D	1.552(7)
C003	C008	1.387(7)	C00B	C00F	1.544(7)
C004	C005	1.398(7)	C00B	C00I	1.543(7)
C004	C006	1.413(7)	C00C	C00J	1.532(7)

Table 2: Continued

C004	C007	1.453(7)	C00C	C00M	1.516(8)
C005	C00A	1.372(7)	C00D	C00E	1.529(7)
C006	C00B	1.537(7)	C00D	C00G	1.554(7)
C007	C00E	1.512(7)	C00F	C00H	1.534(7)
C008	C00A	1.412(7)	C00G	C00K	1.547(8)
C009	C00G	1.533(7)	C00G	C00L	1.538(8)

Table 3: Bond Angles for Sugiol **(1).**

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C008	C003	C006	121.0(5)	C006	C00B	C00I	105.8(4)
C005	C004	C006	120.2(5)	C00F	C00B	C00D	108.9(4)
C005	C004	C007	118.8(5)	C00I	C00B	C00D	115.3(4)
C006	C004	C007	121.0(5)	C00I	C00B	C00F	109.1(4)
C00A	C005	C004	123.1(5)	C00A	C00C	C00J	110.3(4)
C003	C006	C004	117.3(5)	C00M	C00C	C00A	113.0(5)
C003	C006	C00B	121.3(5)	C00M	C00C	C00J	110.7(5)
C004	C006	C00B	121.3(5)	C00B	C00D	C00G	117.1(4)
O001	C007	C004	121.7(5)	C00E	C00D	C00B	110.4(4)
O001	C007	C00E	119.7(5)	C00E	C00D	C00G	113.6(4)
C004	C007	C00E	118.6(4)	C007	C00E	C00D	113.6(4)
O002	C008	C003	121.1(5)	C00H	C00F	C00B	112.9(4)
O002	C008	C00A	116.7(5)	C009	C00G	C00D	108.1(4)
C003	C008	C00A	122.2(5)	C009	C00G	C00K	110.1(4)
C00H	C009	C00G	113.3(4)	C009	C00G	C00L	108.0(4)
C005	C00A	C008	116.2(5)	C00K	C00G	C00D	114.4(4)
C005	C00A	C00C	124.0(5)	C00L	C00G	C00D	108.9(4)
C008	C00A	C00C	119.8(5)	C00L	C00G	C00K	107.1(5)
C006	C00B	C00D	107.4(4)	C009	C00H	C00F	110.9(4)
C006	C00B	C00F	110.3(4)				

(2) Tri(2,4-di-tert-butylphenyl) phosphate:

Table 4: Crystal data and structure refinement for tri(2,4-di-tert-butylphenyl) phosphate **(4).**

Identification code	Tri(2,4-di-tert-butylphenyl) phosphate
Empirical formula	$C_{42}H_{63}O_4P$
Formula weight	662.45
Temperature/K	100.00(13)
Crystal system	monoclinic
Space group	$P2_1/n$
a/Å	15.4779(7)
b/Å	16.1250(7)
c/Å	15.9195(6)
α/°	90
β/°	90.955(4)
γ/°	90
Volume/Å^3	3972.7(3)
Z	111

Table 4: Continued

ρ_{calc}g/cm^3	1.108
μ/mm^{-1}	0.897
F(000)	1448.0
Crystal size/mm^3	0.35 × 0.2 × 0.05
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	7.804 to 150.31
Index ranges	-19 ≤ h ≤ 18, -19 ≤ k ≤ 19, -19 ≤ l ≤ 13
Reflections collected	16593
Independent reflections	7226 [R_{int} = 0.0367, R_{sigma} = 0.0418]
Data/restraints/parameters	7226/0/442
Goodness-of-fit on F^2	1.022
Final R indexes [I>=2σ (I)]	R_1 = 0.0488, wR_2 = 0.1287
Final R indexes [all data]	R_1 = 0.0628, wR_2 = 0.1426
Largest diff. peak/hole / e Å^{-3}	0.55/-0.39

Table 5: Bond Lengths for tri(2,4-di-tert-butylphenyl) phosphate**(4).**

Atom	Atom	Length/Å	Atom	Atom	Length/Å
P1	O1	1.5731(13)	C14	C16	1.390(3)
P1	O2	1.5757(14)	C16	C33	1.537(3)
P1	O3	1.5691(13)	C17	C25	1.401(3)
P1	O4	1.4578(14)	C17	C27	1.538(3)
O1	C20	1.410(2)	C18	C25	1.398(3)
O2	C11	1.411(2)	C18	C26	1.543(3)
O3	C23	1.418(2)	C19	C24	1.527(3)
C5	C8	1.383(3)	C21	C22	1.543(2)
C5	C20	1.383(3)	C21	C28	1.526(3)
C6	C16	1.400(3)	C21	C29	1.536(3)
C6	C22	1.401(3)	C21	C34	1.537(3)
C7	C14	1.393(3)	C22	C23	1.404(3)
C7	C23	1.380(3)	C24	C40	1.525(3)
C8	C19	1.386(3)	C24	C41	1.528(3)
C9	C13	1.390(3)	C24	C43	1.524(3)
C9	C19	1.403(3)	C26	C32	1.544(3)
C10	C13	1.547(3)	C26	C35	1.540(3)
C10	C30	1.538(3)	C26	C39	1.535(3)
C10	C31	1.529(3)	C27	C37	1.518(3)
C10	C38	1.534(3)	C27	C45	1.517(3)
C11	C15	1.379(3)	C27	C46	1.525(4)
C11	C18	1.400(3)	C33	C36	1.533(3)
C12	C15	1.390(3)	C33	C42	1.514(3)
C12	C17	1.384(3)	C33	C44	1.535(3)
C13	C20	1.403(3)			

Table 6: Bond Angles for tri(2,4-di-tert-butylphenyl) phosphate**(4).**

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
O1	P1	O2	100.22(7)	C5	C20	O1	120.50(17)
O3	P1	O1	101.50(7)	C5	C20	C13	121.94(19)

Table 6: Continued

O3	P1	O2	102.07(7)	C13	C20	O1	117.47(16)
O4	P1	O1	117.19(8)	C28	C21	C22	111.75(16)
O4	P1	O2	116.65(8)	C28	C21	C29	107.54(16)
O4	P1	O3	116.52(7)	C28	C21	C34	106.85(16)
C20	O1	P1	127.89(11)	C29	C21	C22	109.44(16)
C11	O2	P1	126.59(12)	C29	C21	C34	110.73(17)
C23	O3	P1	128.69(12)	C34	C21	C22	110.48(16)
C8	C5	C20	120.09(18)	C6	C22	C21	121.65(17)
C16	C6	C22	124.16(18)	C6	C22	C23	115.62(17)
C23	C7	C14	119.76(18)	C23	C22	C21	122.72(17)
C5	C8	C19	121.07(18)	C7	C23	O3	120.85(17)
C13	C9	C19	124.60(18)	C7	C23	C22	122.21(17)
C30	C10	C13	110.68(17)	C22	C23	O3	116.88(16)
C31	C10	C13	110.04(16)	C19	C24	C41	110.81(17)
C31	C10	C30	110.27(18)	C40	C24	C19	108.69(17)
C31	C10	C38	106.77(18)	C40	C24	C41	108.34(19)
C38	C10	C13	111.28(17)	C43	C24	C19	112.20(18)
C38	C10	C30	107.72(18)	C43	C24	C40	108.1(2)
C15	C11	O2	120.89(17)	C43	C24	C41	108.6(2)
C15	C11	C18	121.83(17)	C18	C25	C17	124.53(18)
C18	C11	O2	117.26(16)	C18	C26	C32	109.94(17)
C17	C12	C15	120.77(18)	C35	C26	C18	110.21(16)
C9	C13	C10	122.15(17)	C35	C26	C32	110.60(17)
C9	C13	C20	115.45(17)	C39	C26	C18	111.66(16)
C20	C13	C10	122.39(18)	C39	C26	C32	106.69(17)
C16	C14	C7	121.16(17)	C39	C26	C35	107.65(18)
C11	C15	C12	120.34(18)	C37	C27	C17	109.94(16)
C6	C16	C33	120.43(17)	C37	C27	C46	108.7(2)
C14	C16	C6	117.05(17)	C45	C27	C17	111.55(18)
C14	C16	C33	122.49(17)	C45	C27	C37	108.5(2)
C12	C17	C25	116.89(18)	C45	C27	C46	108.2(2)
C12	C17	C27	122.33(17)	C46	C27	C17	109.89(18)
C25	C17	C27	120.77(18)	C36	C33	C16	111.31(17)
C11	C18	C26	122.80(17)	C36	C33	C44	106.5(2)
C25	C18	C11	115.38(17)	C42	C33	C16	112.34(18)
C25	C18	C26	121.81(17)	C42	C33	C36	108.5(2)
C8	C19	C9	116.84(19)	C42	C33	C44	110.0(2)
C8	C19	C24	122.59(17)	C44	C33	C16	108.05(18)
C9	C19	C24	120.57(17)				

2. NMR spectrums of the isolated Compounds

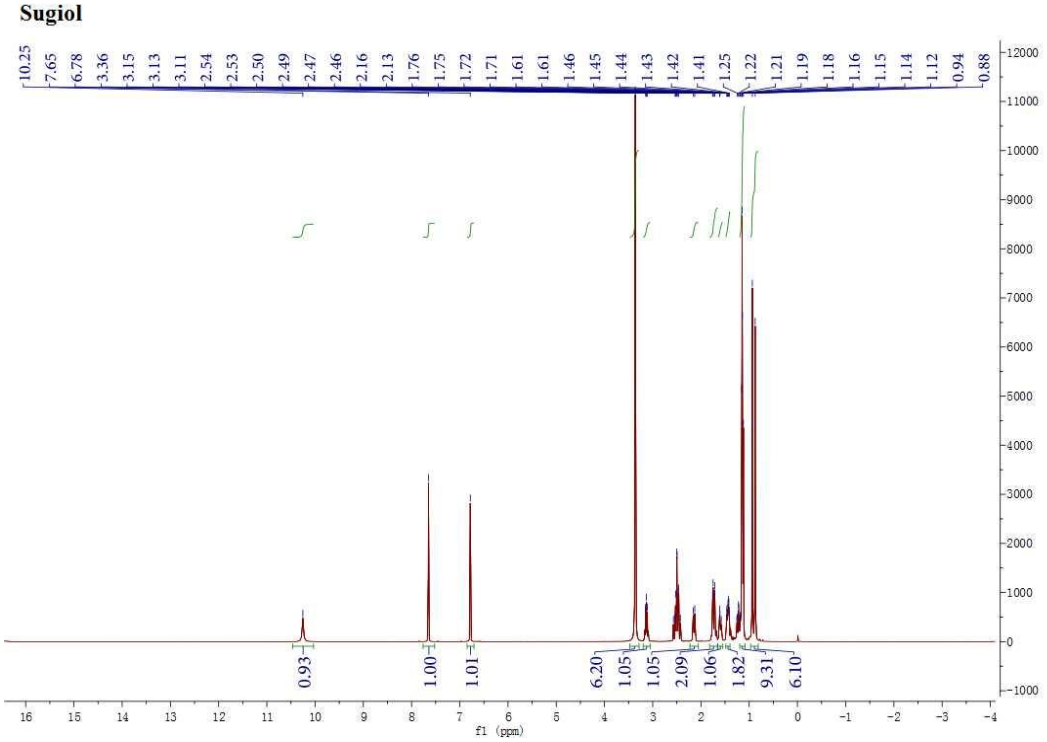


Figure S1.[1]H-NMR spectrum of Sugiol (**1**) in DMSO-d_6.

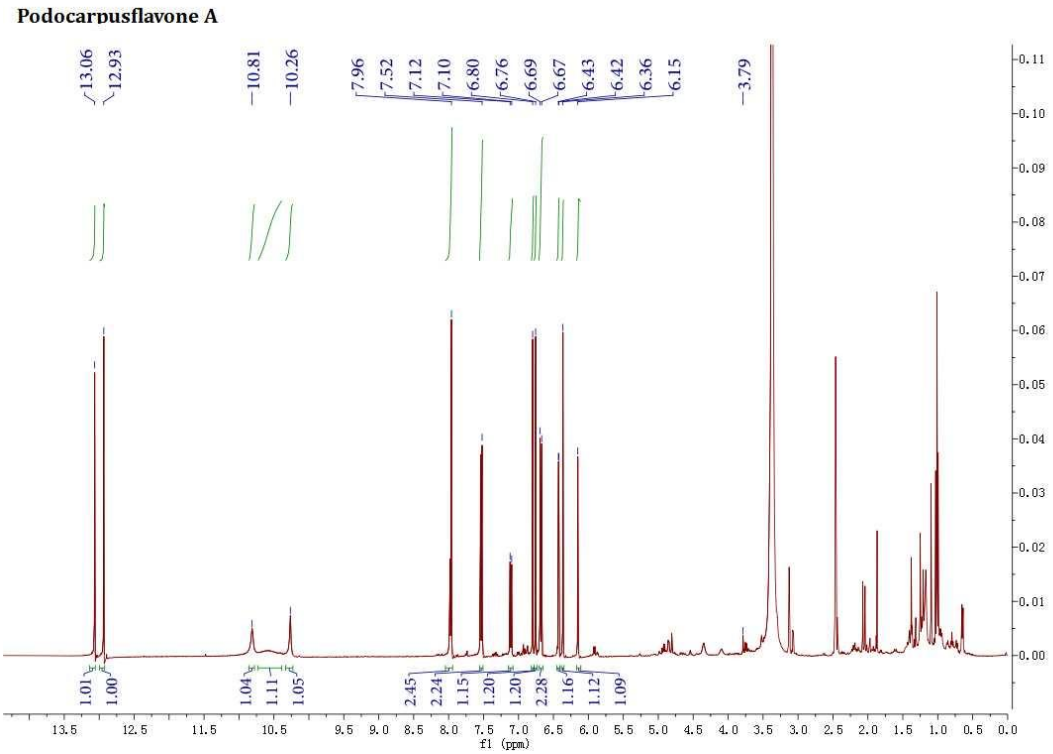


Figure S2: [1]H-NMR spectrum of Podocarpusflavone A(**2**) in DMSO-d_6.

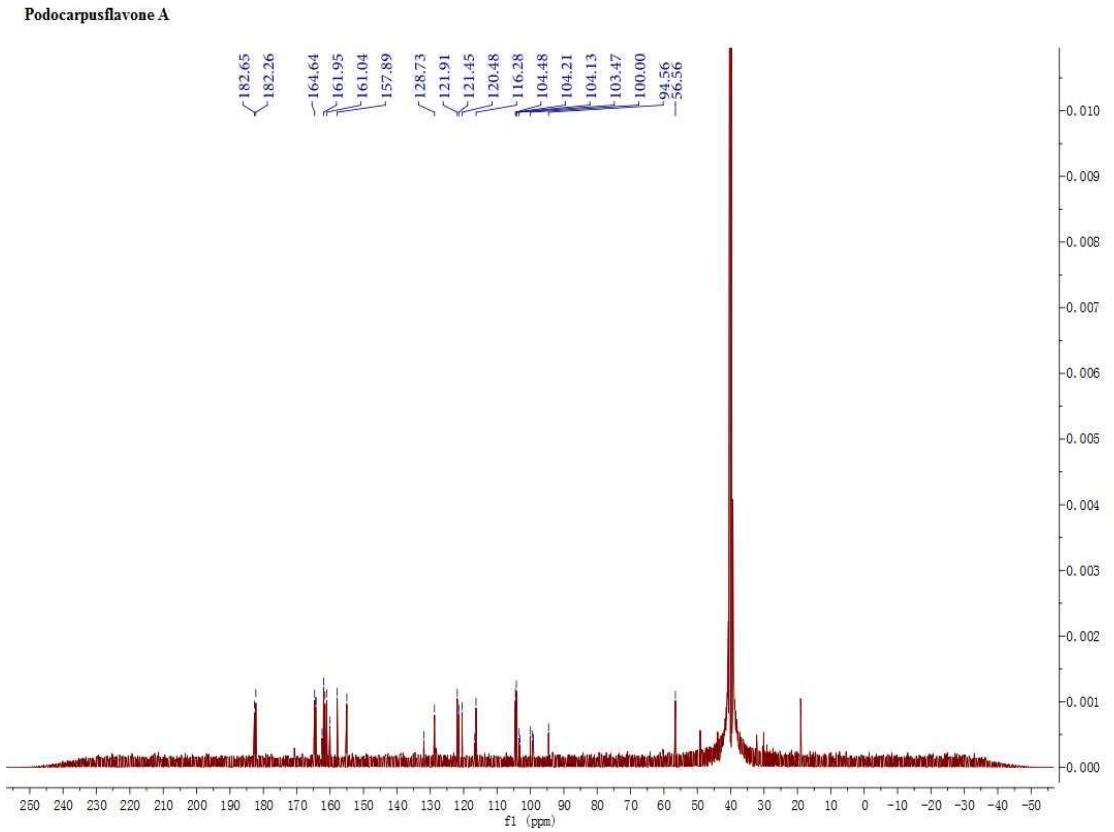


Figure S3. ^{13}C-NMRspectrum ofPodocarpusflavone A (**2**) in DMSO-d_6.

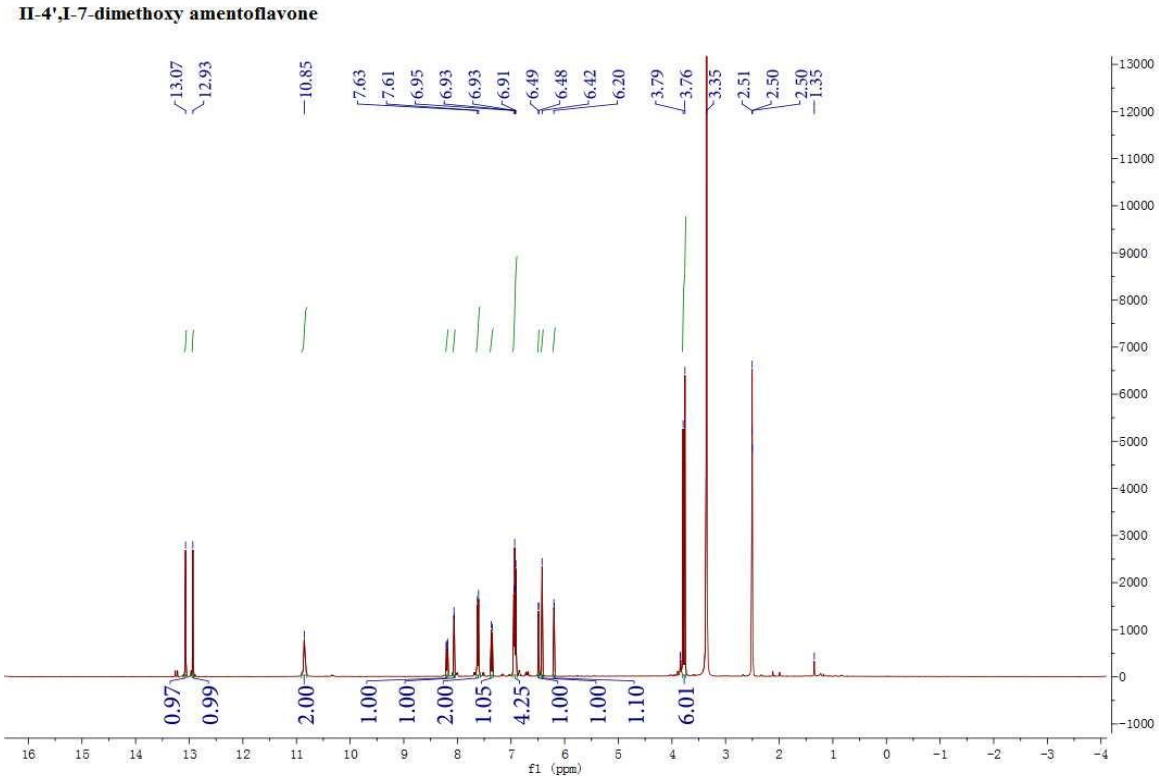


Figure S4: ^{1}H-NMRspectrum ofII-4',I-7-dimethoxy amentoflavone(**3**) in DMSO-d_6.

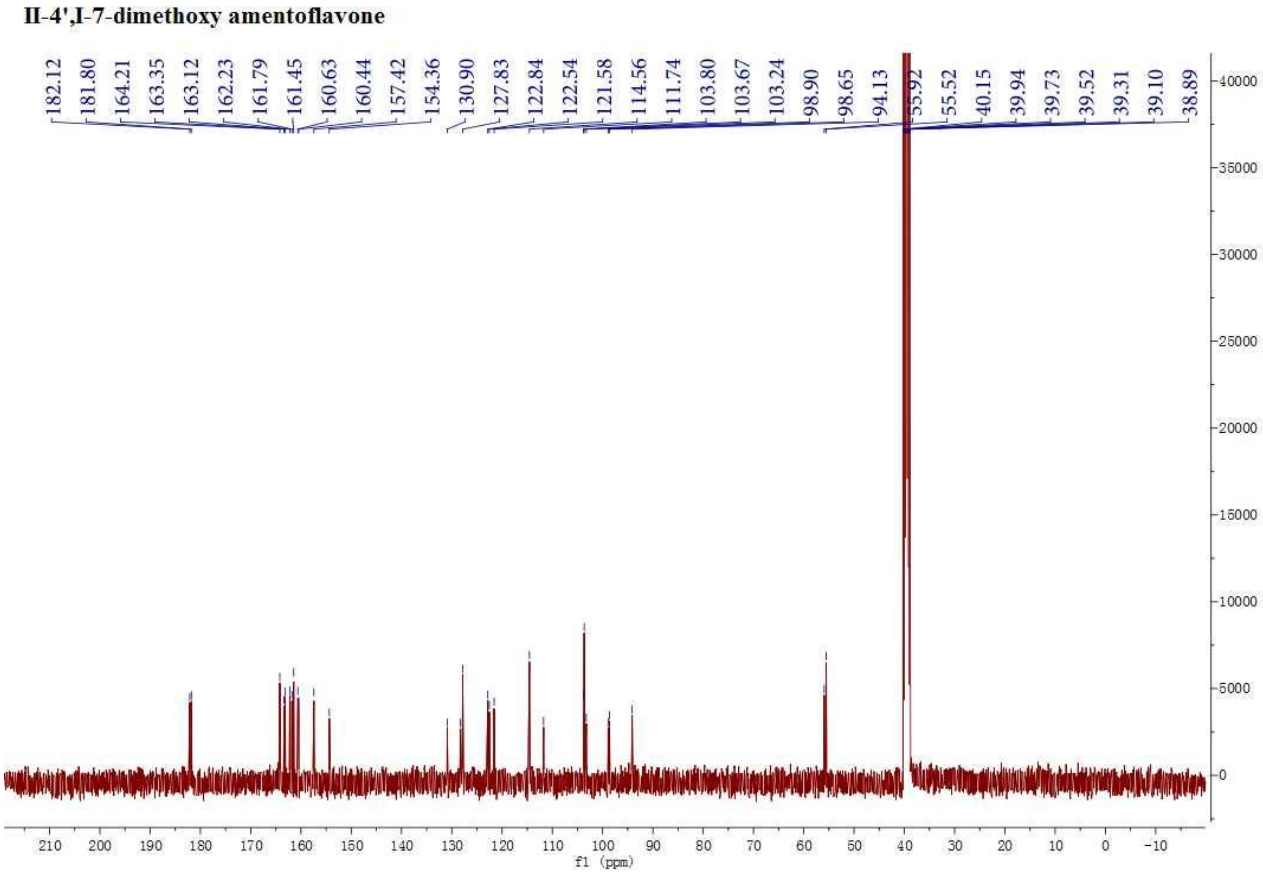
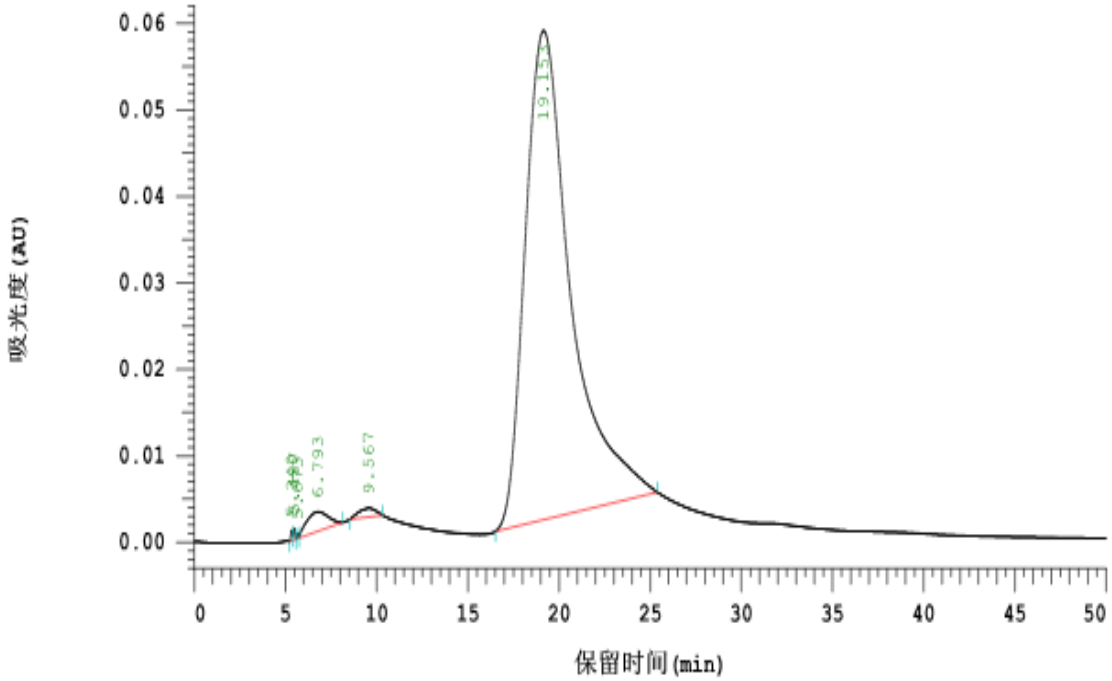


Figure S5: ^{13}C-NMRspectrum ofII-4',I-7-dimethoxy amentoflavone (**3**) in DMSO-d_6.

3. Compounds spectrums of HPLC analysis.

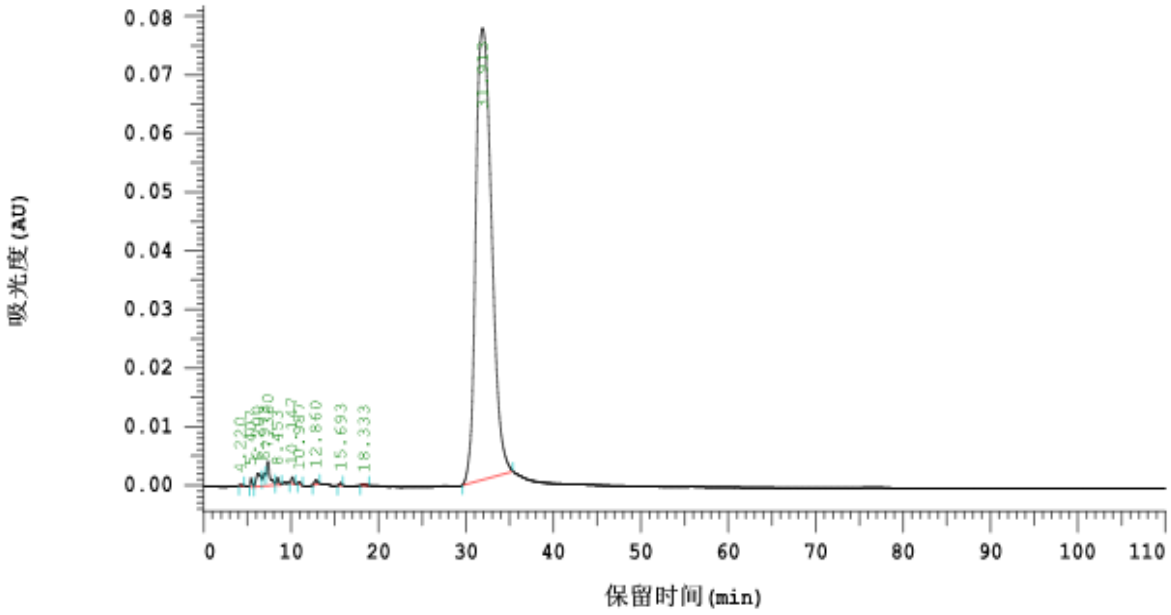


色谱类型：整合色谱，240 - 260 nm

峰的定量：面积
计算方法：面积%

No.	RT	面积	浓度1	BC
1	5.340	3614	0.072	BV
2	5.487	3754	0.074	VB
3	5.673	1674	0.033	BV
4	6.793	92629	1.835	VB
5	9.567	33298	0.660	BB
6	19.153	4911657	97.326	BB
		5046626	100.000	

Figure S6: HPLC spectrum of Sugiol (**1**).

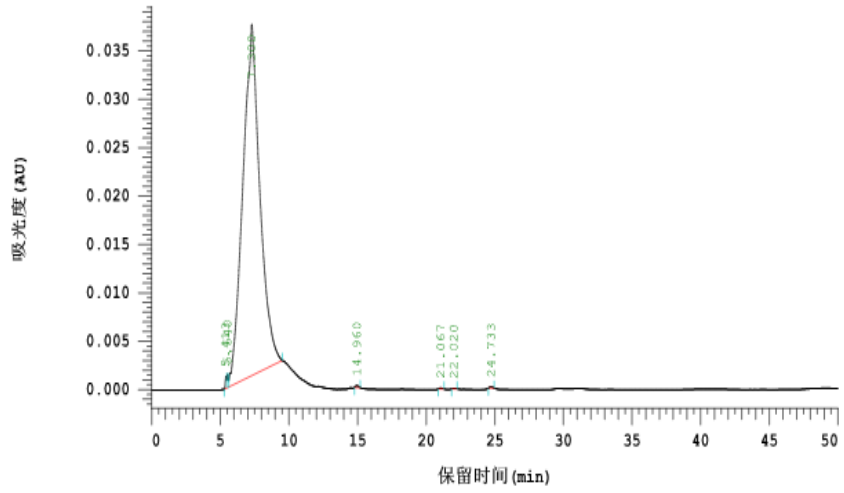


色谱类型：整合色谱，240 - 260 nm

峰的定量：面积
计算方法：面积%

No.	RT	面积	浓度1	BC
1	4.220	1059	0.021	BB
2	5.407	9099	0.184	BB
3	6.200	40595	0.821	BV
4	6.973	22225	0.450	VV
5	7.360	53406	1.080	VV
6	8.453	11298	0.229	VB
7	10.147	8768	0.177	BB
8	10.987	2914	0.059	BB
9	12.860	8372	0.169	BB
10	15.693	3379	0.068	BB
11	18.333	2067	0.042	BB
12	31.913	4780800	96.699	BB
		4943982	100.000	

Figure S7: HPLC spectrum of Podocarpusflavone A (**2**).



色谱类型：整合色谱，240 - 260 nm

峰的定量：面积
计算方法：面积%

No.	RT	面积	浓度1	BC
1	5.413	3530	0.225	BV
2	5.540	4481	0.286	VV
3	7.300	1554724	99.257	VB
4	14.960	1472	0.094	BB
5	21.067	737	0.047	BB
6	22.020	461	0.029	BB
7	24.733	951	0.061	BB
		1566356	100.000	

Figure S8: HPLC spectrum of II-4',I-7-dimethoxy amentoflavone (**3**).

4. Compounds spectrums of HR-MS analysis.

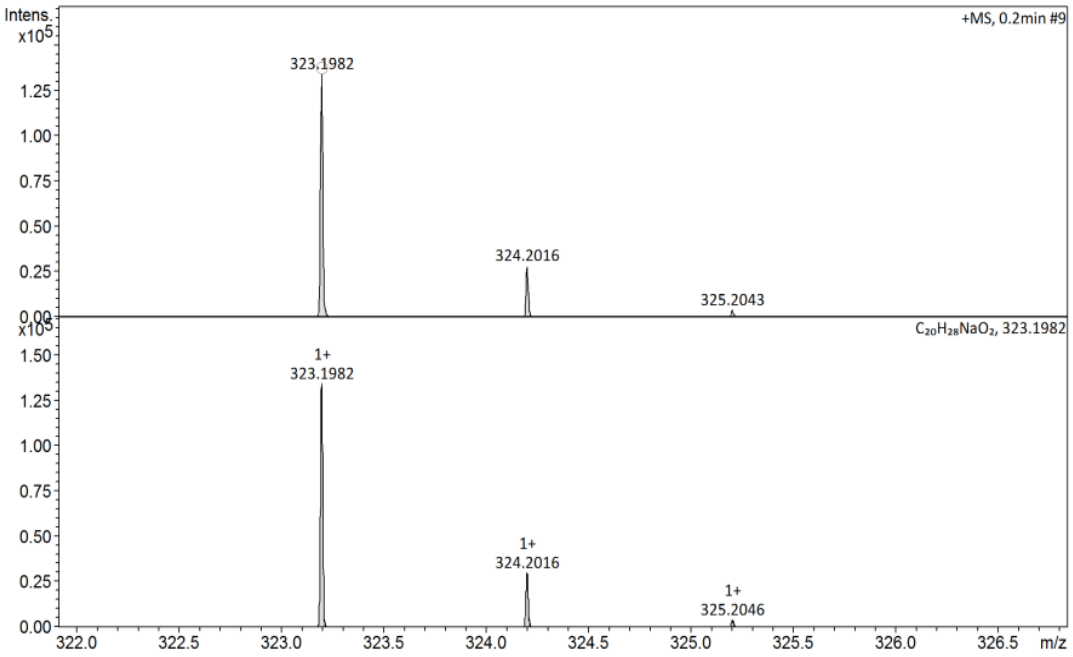


Figure S9: HR-MS spectrum of suigol (**1**).

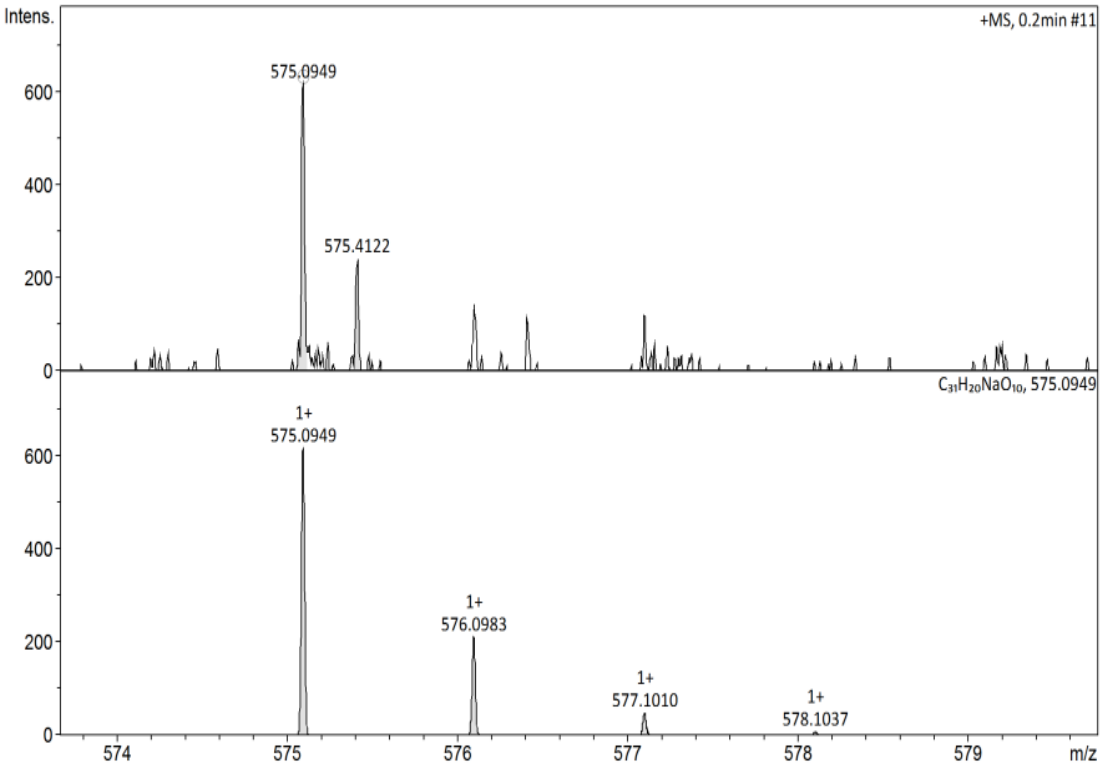


Figure S10: HR-MS spectrum of Podocarpusflavone A (**2**).

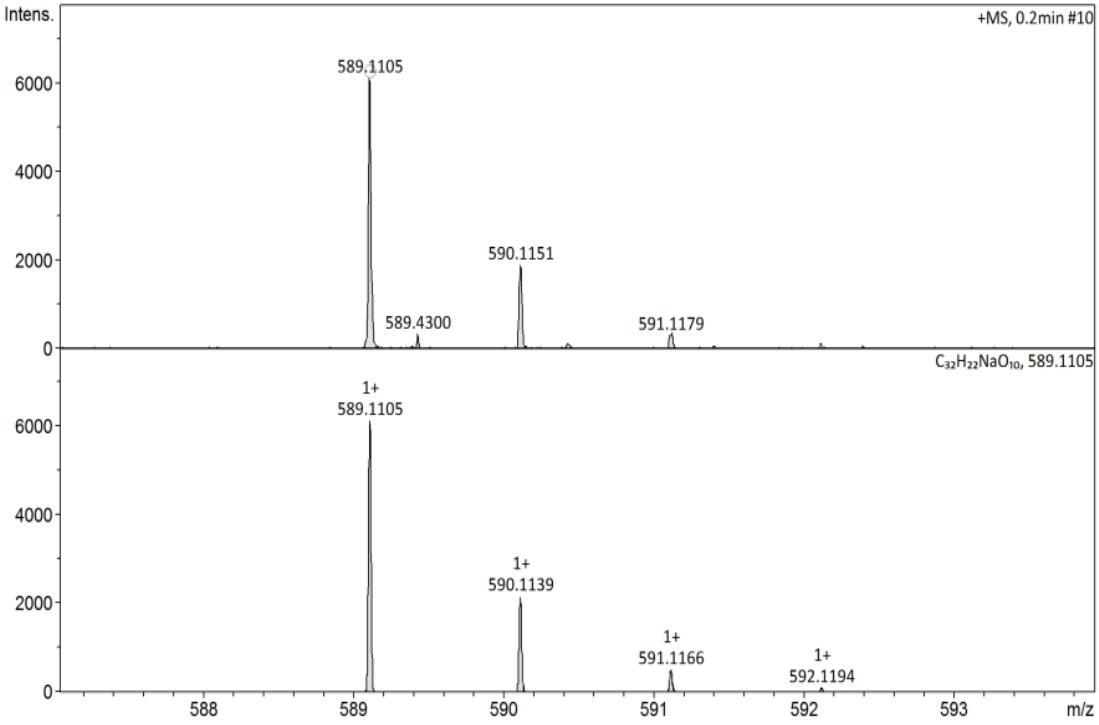


Figure S11: HR-MSspectrum ofII-4',I-7-dimethoxy amentoflavone A (**3**).