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Sesquiterpene Hydroquinones from the Sponge *Reniera Mucosa*

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Abstract: The sponge *Reniera mucosa* contains eight new compounds, six of them of the panicein class (**1-6**) and two novel cyclohexenones renierin A (**7**) and renierin B(**8**), together with seven related known compounds (**9-15**). Cyclohexenones **7** and **8** might be the biosynthetic intermediates from farnesyl precursors to paniceins. The structures were elucidated by interpretation of spectral data and chemical interconversions. Four of the new compounds (**2**, **3**, **4** and **6**) showed significant *in vitro* cytotoxicity.

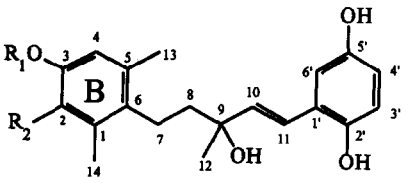
Paniceins are a group of marine natural compounds characterized for possessing an aromatic sesquiterpenoid moiety linked to an unsubstituted benzoquinol or benzoquinone. They also occur as chromenols, the cyclic isomers of the corresponding quinols and were isolated from *Halichondria panicea*.[2]

Sponges of the genus *Reniera* produce aryl carotenoids, isoquinoline quinones, pentacyclic alkaloids and diacetylene metabolites.[3] In 1993 Minale and coworkers reported, from specimens of *R. fulva*, the isolation of four known paniceins together with the new fulvanin 1 and fulvanin 2. This latter compound is a non aromatic cyclic sesquiterpenoid linked to a hydroquinone moiety.[4]

In the course of our investigations directed towards the search of new biologically active compounds of marine organisms we obtained specimens of the sponge *Reniera mucosa* collected near Tarifa Island (Spain). The chemical study of *R. mucosa* has led to the isolation and characterization of eight new compounds, six of them of the panicein class (**1-6**) and two related cyclohexenones named renierins A (**7**) and B (**8**) together with the known panicein A (**9**), the corresponding hydroquinone (**10**), panicein B_2 (**11**), panicein B_3 (**12**), panicein C (**13**),[2] fulvanin 1 (**14**) and fulvanin 2 (**15**).[4]

Specimens of *Reniera mucosa* were collected by hand using SCUBA and immediately frozen. The non polar material from an acetone extract was chromatographed on Si gel. Further purification using normal phase HPLC allowed the isolation of the following compounds: panicein D (**1**, 0.030% dry wt), panicein E (**2**, 0.013 % dry wt), panicein A_2 (**3**, 0.028% dry wt), panicein F_1 (**4**, 0.020% dry wt), panicein G (**5**, 0.003% dry wt), panicein F_2 (**6**, 0.003% dry wt), renierin A (**7**, 0.006% dry wt) and renierin B (**8**, 0.004% dry wt) together with the seven other known compounds (**9-15**) above mentioned.

Panicein D (**1**) was the major compound obtained from *R. mucosa* and was isolated as an orange oil. The molecular formula, $C_{21}H_{24}O_5$, was obtained from the high resolution mass measurement. The aromatic proton signals at δ 6.52 (dd,J=8.5 and 2.8,1H), 6.62 (d,J=8.5,1H) and 6.86 (d,J=2.8,1H) that were assigned to a monosubstituted hydroquinone together with the aromatic methyl signals at δ 2.31 (s,3H) and 2.54 (s,3H)



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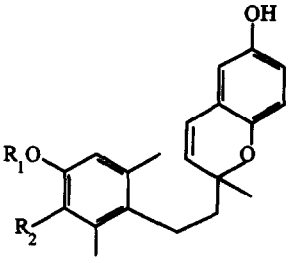
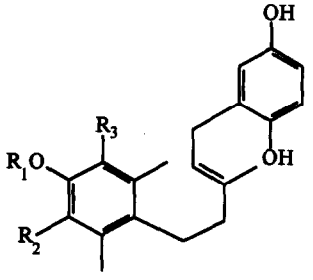


3 R_1= CH_3 R_2= CH_3
6 R_1= CH_3 R_2= CH_2OH
11 R_1= H R_2= CHO



4 R_1= CH_3 R_2= CH_2OH R_3= H
5 R_1= H R_2= CH_2OH R_3= H
10 R_1= CH_3 R_2= CH_3 R_3= H
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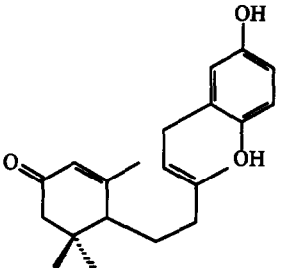
13 R_1= H R_2= CHO R_3= OH



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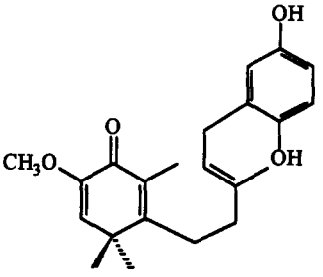

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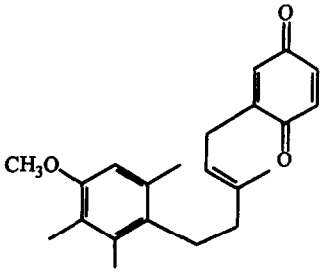

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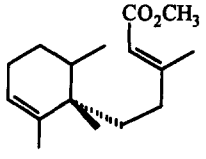

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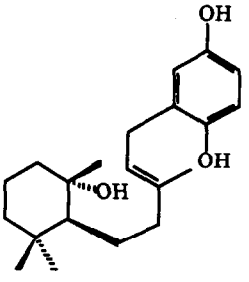

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supported that **1** had a structure closely related to paniceins. Comparison of the ^{1}H NMR data of **1** with those reported[2] for panicein B_3 (**12**) indicated that **1** lack one benzylic methylene and the vinylic methyl group presenting instead a conjugated E double bond indicated by the two doublets at δ 6.33 (d,J=16.5,1H) and 6.90 (d,J=16.5,1H), that were correlated to the ^{13}C NMR doublets at δ 135.8 and 123.2 on the XHCORR experiment, and a tertiary alcohol bearing a methyl group as ascertained by the signal on the ^{13}C NMR spectrum at δ 73.7 (s) and the ^{1}H NMR singlet at δ 1.44 (s,3H). The ^{1}H NMR signal at δ 10.34 (s,1H) together with the doublet on the ^{13}C NMR spectrum at δ 195.6 indicated the presence of an aldehyde. The substitution pattern of the B ring on panicein D (**1**) was deduced from a series of nuclear Overhauser effect difference spectroscopy experiments, in particular, the enhancements produced on Me-13 and Me-14 upon irradiation of the benzylic methylene signal and the enhancements observed on H-4 and the aldehyde proton signals upon irradiation of Me-13 and Me-14 proton signals, respectively. It was therefore proposed structure **1** for panicein D.

Panicein E (**2**) was isolated as a yellow oil. The molecular formula, $C_{22}H_{28}O_4$, was obtained from the high resolution mass measurement. Comparison of spectral data of panicein D (**1**) with those of panicein E (**2**) strongly suggested that the structures of both compounds were quite similar. The differences were clearly located on the pentasubstituted aromatic ring. The ^{1}H NMR spectrum of panicein E contained four singlets at δ 2.07 (s,3H), 2.19 (s,3H), 2.27 (s,3H) and 3.73 (s,3H) that were assigned to three methyl and one methoxy substituents of the pentasubstituted aromatic nucleus and, in turn, lack the aldehyde proton signal. These data defined the structure **2** for panicein E. The proposed structure was confirmed by a series of NOEDS experiments that provided information about the substitution pattern of ring B. Irradiation of the benzylic methylene signal at δ 2.66 (m,2H) caused enhancements on both Me-13 and Me-14 signals whereas irradiation of the Me-13 and Me-14 signals resulted in enhancement of H-4 and Me-15, respectively.

Panicein A_2 (**3**) was isolated as a colorless oil. The molecular formula $C_{22}H_{26}O_3$ was obtained from the high resolution mass measurement. The ^{1}H NMR spectrum signal at δ 6.35 (d,J=9.8,1H) and 5.71 (d,J=9.8,1H) that were correlated to the doublets at δ 123.6 and 130.6 respectively on the XHCORR experiment indicated the presence of a conjugated double bond. The ^{1}H NMR signals at δ 6.60 (d,J=8.6,1H), 6.54 (dd,J=8.6 and 2.8, 1H) and 6.46 (d,J=2.8,1H) indicated the presence of a 1,2,4-trisubstituted aromatic ring. The six signals remaining on the unsaturated zone of the ^{13}C NMR spectrum [110.7 (d), 122.6 (s), 130.9 (s), 133.6 (s), 135.4 (s), 155.3 (s)] account for another aromatic ring which must be methoxy-tri-methyl substituted as indicated by the ^{1}H NMR signals at δ 2.06 (s,3H), 2.13 (s,3H), 2.22 (s,3H), 3.72 (s,3H) and 6.53 (s,1H). These data could be accommodated by structure **3**. The substitution pattern of the pentasubstituted aromatic ring of panicein A_2 (**3**) was defined by a series of NOEDS experiments: irradiation of the benzylic methylene signal caused enhancements on Me-13 and Me-14 signals, irradiation of Me-13 signal resulted in enhancement of the H-4 signal and irradiation of the Me-15 signal produced enhancement of the Me-14 signal. The known compound panicein A hydroquinone (**10**) was converted into panicein A_2 (**3**) on treatment with K_2CO_3 in

refluxing acetone.[5]

Panicein F_1 (**4**) was obtained as a pale orange oil of molecular formula $C_{22}H_{28}O_4$. The spectral data recorded showed great similarities with those reported for panicein A hydroquinone (**10**).[4] The main difference was a singlet at δ 4.68 (s,2H) on the ^1H NMR spectrum together with a triplet at δ 56.3 on the ^{13}C NMR spectrum that were assigned to a primary alcohol. Since both ^1H NMR and ^{13}C NMR spectra accounted for two aromatic methyl groups it was proposed the structure of alcohol **4** for panicein F_1. A series of NOEDS experiments provided confirmation to the substitution of ring B. Irradiation of the Me-13 signal caused enhancement of H-4 and H-7 protons and irradiation of the Me-14 signal resulted in enhancement of the methylene protons signals at C-15 and C-7 .

Panicein G (**5**) was isolated as an amorphous powder. The molecular formula $C_{21}H_{26}O_4$ was deduced from the high resolution mass measurement. Comparison of ^1H NMR and ^{13}C NMR data of panicein G (**5**) with those of panicein F_1 (**4**), in particular the absence of the singlet at δ 3.77 (s,3H) due to the methoxy group of panicein F_1 (**4**), clearly indicated that **5** was the corresponding demethyl derivative of **4**. It was therefore proposed the structure **5** for panicein G. The known compound panicein B_3 (**12**) was converted into panicein G (**5**) by lithium aluminum hydride reduction to confirm the proposed structural assignments.

Panicein F_2 (**6**) was obtained as a yellowish oil. The molecular formula, $C_{22}H_{26}O_4$, indicated one more degree of insaturation than that of panicein F_1 (**4**). Both ^1H NMR and ^{13}C NMR data indicated that **6** contained a pentasubstituted aromatic ring identical to that of panicein F_1 (**4**). The ^1H NMR spectrum of **6** lacked the signals corresponding to the benzylic methylene and the vinylic methyl group and presented, in addition, two doublets at δ 5.72 (d,J=9.8,1H) and 6.35 (d,J=9.8,1H) that were assigned to a Z double bond. Furthermore a singlet at δ 1.41 (s,3H) together with the ^{13}C NMR signals at δ 26.2 (q) and 79.0 (s) were consistent with a methyl group attached to a fully substituted carbon bearing oxygen. These spectral data strongly suggest that panicein F_2 (**6**) was the corresponding chromenol of **4**.[5] Panicein F_2 (**6**) was obtained upon treatment of hydroquinone **4** with K_2CO_3 in refluxing acetone.

Renierin A (**7**) was isolated as a yellowish oil. The molecular formula $C_{21}H_{28}O_3$ was obtained from the high resolution mass measurement. The ^1H NMR spectrum showed the signals of a monosubstituted hydroquinone system 6.43 (dd,J=8.7 and 3.0,1H), 6.52 (d, J=3.0,1H) and 6.57 (d,J=8.7,1H). Both ^1H NMR and ^{13}C NMR indicated similarities with panicein F_1 (**4**) and G (**5**) but ring B in renierin A (**7**) was not aromatic. The ultraviolet absorption at 245 nm (ε 7550) and the IR band at 1690 cm^{-1} were assigned to a cyclohexenone moiety. The ^{13}C NMR signals at δ 24.8 (q), 125.2 (d), 170.1 (s) and 202.3 (s) and the ^1H NMR signals at δ 1.72 (s,3H) and 5.77 (s,1H) were due to a β-methyl-α,β-unsaturated carbonyl system. Analysis of the COSY spectrum showed that two doublets at δ 1.96 (d,J=17.2,1H) and 2.44 (d,J=17.2,1H) were exclusively mutually coupled and were assigned to an isolated methylene group. Two singlets δ 0.98 (s,3H) and 1.07 (s,3H) indicated the presence of a *gem*-dimethyl group attached to a quaternary carbon. These data defined the trimethylcyclohexenone ring in renierin A (**7**).

Renierin B (**8**) was isolated as a yellowish oil. The molecular formula $C_{22}H_{28}O_4$ was deduced from the high resolution mass measurement and required one more degree of unsaturation than that of renierin A (**7**). A similar rationale as followed for **7** indicated that **8** contained the monosubstituted hydroquinone moiety and lack aromaticity on ring B. The ultraviolet absorption at 240 nm confirmed the presence of an α,β-unsaturated ketone. This UV data together with the ^{13}C NMR signals at δ 41.1 (s), 120.8 (d), 134.2 (s), 144.6 (s), 173.3 (s) and 196.2 (s) indicated a cross conjugated cyclohexadienone ring. The substitution of this ring was defined as follows: a signal at δ 2.35 (m,2H) was assigned to the allylic methylene protons at C-7, two singlets at δ 1.21 (s,6H) and 1.97 (s,3H) were due to a *gem*-dimethyl group attached to a quaternary carbon and a vinylic methyl group, respectively, a singlet at δ 3.67 (s,3H) indicated a methoxy substituent and a singlet at δ 6.24 (s,1H) was assigned to an olefinic proton β to the carbonyl group. With the data above considered, two possibilities of substitution emerge for the cyclohexadienone ring: 1,1,5-trimethyl-3-methoxy or the alternative 1,1,3-trimethyl-5-methoxy. This latter possibility can be eliminated, not only upon biogenetic considerations, but by the presence of a COSY cross peak for a long-range coupling between Me-13 and C-7 methylene protons signals. It was therefore proposed structure **8** for renierin B.

It has been suggested[2,6] that the aromatic group of terpenoid origin of paniceins might arise by cyclization of the farnesyl precursor to an abscisane derivative followed by a 1,2-methyl migration and subsequent oxidation. The co-occurrence of paniceins (**1-6, 10-12**) and renierin A (**7**) in *Reniera mucosa* supports this biosynthetic hypothesis. Renierin B (**8**) seems to be a biosynthetic intermediate to more oxidized derivatives like panicein C (**13**).

The new compounds isolated from *Reniera mucosa* were tested against P388 mice lymphoma, A549 human lung carcinoma, HT29 human colon carcinoma and MEL28 human melanoma cell lines to detect *in vitro* cytotoxicity. Only results of ED_{50} under 10 μg/mL will be reported. Both panicein D (**1**) and panicein A_2 (**3**) showed cytotoxicity to the four cell lines above mentioned (ED_{50}= 5μg/mL). Panicein E (**2**) showed stronger activity to P388 and MEL20 cell lines (ED_{50}= 2.5μg/mL) than to A549 and HT29 (ED_{50}= 5μg/mL). Panicein F_1 (**4**) exhibited cytotoxicity against P388, A549 and MEL20 (ED_{50}= 5μg/mL). Furthermore this latter compound **4** showed mild inhibition activity on screens for DHFR (dihydrofolate reductase) inhibition (ED_{50}= 3μg/mL).

EXPERIMENTAL

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared spectra were recorded in a Perkin-Elmer 881 spectrophotometer. ^1H-NMR and ^{13}C-NMR spectra were made on a Varian 400 at 400 MHz and 100 MHz respectively using methanol-d_4 as solvent. The resonances of residual methanol at δ_H 3.30 and δ_C 49.0 were used as internal reference for ^1H and ^{13}C spectra, respectively. An asterisk indicates interchangeable signals. UV spectra were recorded on a Phillips PU 8710 spectrophotometer. Mass spectra were recorded on a VG 12250 or on Kratos MS 80RFA spectrometers. In High Performance Liquid Chromatography separations LiChrosorb silica 60 was used in normal phase mode using a differential

refractometer and a UV detectors. All solvents were distilled from glass prior to use.

Extraction and isolation: The sponge *Reniera mucosa* (70.52 g, dry weight) was collected by hand using SCUBA near Tarifa Island in July 1993 and was immediately frozen. The frozen tissue was extracted exhaustively with acetone at room temperature. The filtered Me_2CO solution was evaporated under reduced pressure and the aqueous residue was extracted with Et_2O. The solvent was evaporated to give an oil residue (4 g) which was chromatographed on a SiO_2 column using solvents of increasing polarity from hexane to diethyl-ether and subsequently chloroform-methanol. Selected non polar fractions contained fulvanin 1 (**14**, 140 mg, 0,199 % dry weight), panicein A_2 (**3**, 20 mg, 0.028 % dry weight), a mixture of panicein B_2 (**11**, 44 mg, 0,062 % dry weight), and panicein A hydroquinone (**10**, 75 mg, 0,106 % dry weight) that was further separated by HPLC, panicein B_3 (**12**, 65 mg, 0.092% dry weight) and panicein C (**13**, 70 mg, 0.100% dry weight). The study of more polar factions in HPLC afforded, in order of increasing polarity, fulvanin 2 (**15**, 12 mg, 0,017 dry weight), renierin A (**7**, 4 mg, 0.006 % dry weight), panicein F_2 (**6**, 2 mg, 0.003 % dry weight), panicein E (**2**, 9 mg, 0.013 % dry weight), renierin B (**8**, 3 mg, 0.004 % dry weight), panicein F_1 (**4**, 14 mg, 0.020 % dry weight), panicein G (**5**, 2 mg, 0.003 % dry weight) and panicein D (**1**, 21 mg, 0.030 % dry weight).

Panicein D (1): Orange oil; $[\alpha]_D$= -8.0° (c= 0.14, $CHCl_3$); **IR** (film) 3500-3100, 1640, 1580, 1460 cm^{-1}; **UV** ($CHCl_3$) λ_{max} 242 (ε=17560), 274 (ε=17960) nm; **^{1}H-NMR** (methanol-d_4) δ 10.34 (s, 1H, H-15), 6.90 (d, 1H, J= 16.5, H-11), 6.86 (d, 1H, J=2.8, H-6'), 6.62 (d, 1H, J=8.5, H-3'), 6.58 (s, 1H, H-4), 6.52 (dd, 1H, J=8.5, 2.8, H-4'), 6.33 (d, 1H, J=16.5, H-10), 2.70 (m, 2H, H-7), 2.54 (s, 3H, H-14), 2.31 (s, 3H, H-13), 1.67 (m, 2H, H-8), 1.44 (s, 3H, H-12); **^{13}C-NMR** (methanol-d_4) δ 195.6 (d, C-15), 161.0 (s, C-3), 152.3 (s, C-5)*, 150.0 (s, C-2')*, 148.0 (s, C-5'), 139.9 (s, C-1), 135.8 (d, C-10), 131.5 (s, C-6), 125.3 (s, C-1'), 123.2 (d, C-11), 117.9 (s, C-2), 116.8 (d, C-4), 116.6 (d, C-3'), 115.4 (d, C-4'), 112.8 (d, C-6'), 73.7 (s, C-9), 43.3 (t, C-8), 28.3 (q, C-12), 24.7 (t, C-7), 21.8 (q, C-13), 14.3 (q, C-14); **EIMS** (70 eV) *m/z* 356 (3), 338 (26), 163 (42), 162 (26), 161 (100); **HREIMS** Obsd. *m/z*= 356.1642 $(M)^+$, $C_{21}H_{24}O_5$ requires *m/z*= 356.1624.

Panicein E (2): Yellow oil; $[\alpha]_D$= -8.4° (c= 0.11, $CHCl_3$); **IR** (film) 3500-3200, 1595, 1460 cm^{-1}; **UV** ($CHCl_3$) λ_{max} 244 (ε=8010) nm; **^{1}H-NMR** (methanol-d_4) δ 6.89 (d, 1H, J= 16.2, H-11), 6.87 (d, 1H, J=2.8, H-6'), 6.62 (d, 1H, J=8.6, H-3'), 6.54 (s, 1H, H-4), 6.52 (dd, 1H, J=8.6,2.8, H-4'), 6.33 (d, 1H, J=16.2, H-10), 3.73 (s, 3H, -OCH_3), 2.66 (m, 2H, H-7), 2.27 (s, 3H, H-13), 2.19 (s, 3H- H-14), 2.07 (s, 3H, H-15), 1.65 (m, 2H, H-8), 1.43 (s, 3H, H-12); **^{13}C-NMR** (methanol-d_4) δ 156.5 (s, C-3), 151.2 (s, C-2'), 149.1 (s, C-5'), 137.0 (d, C-10), 136.5 (s, C-1), 134.6 (s, C-5), 132.2 (s, C-6), 126.3 (s, C-1'), 123.8 (d, C-11), 123.4 (s, C-2), 117.4 (d, C-3'), 116.1 (d, C-4'), 113.4 (d, C-6'), 111.4 (d, C-4), 74.1 (s, C-9), 55,9 (q, -OCH_3), 43.7 (t, C-8), 27.8 (q, C-12), 25.3 (t, C-7), 20.5 (q, C-13), 15.9 (q, C-14), 12.1 (q, C-15); **EIMS** (70 eV) *m/z* 356 (3), 338 (10), 164 (19), 163 (82), 162 (29), 161 (100); **HREIMS** Obsd. *m/z*= 356.1996 $(M)^+$, $C_{22}H_{28}O_4$ requires *m/z*= 356.1988.

Panicein A_2 (3): Colorless oil; **IR** (film) 3300-3100, 1575, 1420 cm^{-1}; **UV** ($CHCl_3$) λ_{max} 242 (ε=5320) nm; **^{1}H-NMR** (methanol-d_4) δ 6.60 (d, 1H, J= 8.6, H-3'), 6.54 (dd, 1H, J=8.6 , 2.8, H-4'), 6.53 (s, 1H, H-4), 6.46 (d, 1H, J=2.8, H-6'), 6.35 (d, 1H, J=9.8, H-11), 5.71 (d, 1H, J=9.8, H-10), 3.72 (s, 3H, -OCH_3), 2.71 (m, 2H, H-7), 2.22 (s, 3H, H-13), 2.13 (s, 3H, H-14), 2.06 (s, 3H, H-15), 1.68 (m, 2H, H-8), 1.40 (s, 3H, H-12); **^{13}C-NMR** (methanol-d_4) δ 155.3 (s, C-3), 150.9 (s, C-2'), 146.4 (s, C-5'), 135.4 (s, C-1), 133.6 (s, C-5), 130.9 (s, C-6), 130.6 (d, C-10), 123.3 (d, C-11), 122.6 (s, C-2)*, 122.3 (s, C-1')*, 116.7 (d, C-3'), 115.6 (d, C-4'), 113.0 (d, C-6'), 110.7 (d, C-4), 78.7(s, C-9), 55.8 (q, -OCH_3), 41.5 (t, C-8), 26.5 (q, C-12), 25.2 (t, C-7), 20.8 (q,

C-13), 16.2 (q, C-14), 12.5 (q, C-15); **EIMS** (70 eV) *m/z* 338 (25), 177 (19), 163 (35), 162 (29), 161 (100); **HREIMS** Obsd. *m/z*= 338.1872 $(M)^+$, $C_{22}H_{26}O_3$ requires *m/z*= 338.1882.

Panicein F_1 (4): Pale orange oil; **IR** (film) 3500-3100, 1600, 1450 cm^{-1}; **UV** ($CHCl_3$) λ_{max} 242 (ε=7070), 288 (ε=7250) nm; **^{1}H-NMR** (methanol-d_4) δ 6.62 (s, 1H, H-4), 6.58 (d, 1H, J=8.6, H-3'), 6.54 (d, 1H, J=3, H-6'), 6.44 (dd,1H, J=8.6, 3.0, H-4'), 5.38 (bt,1H, J= 7.3, H-10), 4.68 (s, 2H, H-15), 3.77 (s, 3H, -OCH_3), 3.26 (d, 2H, J=7.3, H-11), 2.72 (m, 2H, H-7), 2.35 (s, 3H, H-14), 2.30 (s, 3H, H-13), 2.10 (m, 2H, H-8), 1.80 (s, 3H, H-12); **^{13}C-NMR** (methanol-d_4) δ 157.1 (s, C-3), 151.1 (s, C-2'), 148.9 (s, C-5'), 137.9 (s, C-1 and C-9)*, 137.1 (s, C-5)*, 132.7 (s, C-6), 130.1 (s, C-1'), 126.0 (s, C-2), 124.1 (d, C-10), 117.1 (d, C-6'), 116.5 (d, C-3'), 113.9 (s, C-4'), 111.7 (d, C-4), 56.3 (t, C-15), 56.0 (q, -OCH_3), 40.7 (t, C-8), 29.8 (t, C-7), 29.2 (t, C-11), 20.7 (q, C-13), 16.3 (q, C-12), 15.2 (q, C-14); **EIMS** (70eV) *m/z* 356 (1), 338 (31), 215 (46), 179 (81), 177 (10), 164 (25), 163 (100) 162 (57), 161 (99); **HREIMS** Obsd. *m/z*= 354.1828 $(M-2)^+$, $C_{22}H_{26}O_4$ requires *m/z*= 354.1831; *m/z*= 338.1897 $(M-18)^+$, $C_{22}H_{26}O_3$ requires *m/z*= 338.1882.

Panicein G (5): amorphous powder; **IR** (film) 3500-3100, 1600, 1450 cm^{-1}; **UV** ($CHCl_3$) λ_{max} 240 (ε=2040), 290 (ε=1540) nm; **^{1}H-NMR** (methanol-d_4) 6.58 (d ,1H, J=8.6, H-3'), 6.54 (d, 1H, J=3.0, H-6'), 6.47 (s, 1H, H-4), 6.44 (dd, 1H, J= 8,6, 3.0, H-4'), 5.39 (bt, 1H, J= 7.3, H-10), 4.71 (s, 2H, H-15), 3.26 (d, 2H, J= 7.3, H-11), 2.69 (m, 2H, H-7), 2.31 (s, 3H, H.14), 2.23 (s, 3H, H-13), 2.09 (m, 2H, H.8), 1.80 (s, 3H, H-12); **^{13}C-NMR** (methanol-d_4) δ 154.7 (s, C-3), 151.1 (s, C-2'), 149.0 (s, C-5'), 137.6 (s, C-1)*, 137.5 (s, C-9)*, 137.3 (s, C-5)*, 131.6 (s, C-6), 130.1 (s, C-1'), 124.0 (s, C-2), 123.9 (d, C-10), 117.1 (d, C-4), 116.5 (d, C-6'), 115.8 (d, C-3'), 113.9 (d, C-4'), 57.3 (t, C-15), 40.9 (t, C-8), 29.8 (t, C-7), 29.2 (t, C-11), 20.4 (q, C-13), 16.3 (q, C-12), 15.2 (q, C-14); **EIMS** (70 eV) *m/z* 324 (9), 201 (26), 179 (35), 163 (54), 162 (18) 161 (100); **HREIMS** Obsd. *m/z*= 324.1706 $(M-18)^+$, $C_{21}H_{24}O_3$ requires *m/z*= 324.1725; *m/z*= 312.1721 $(M-30)^+$, $C_{20}H_{24}O_3$ requires *m/z*= 312.1726.

Panicein F_2(6): Yellowish oil; **IR** (film) 3500-3200, 1600, 1460 cm^{-1}; **UV** ($CHCl_3$) λ_{max} 243 (ε=7990) nm; **^{1}H-NMR** (methanol-d_4) δ 6.61 (d, 1H, J= 8.6, H-3'), 6.60 (s, 1H, H-4), 6.54 (dd, 1H, J= 8.6, 2.8, H-4'), 6.46 (d, 1H, J= 2.8, H-6'), 6.35 (d, 1H, J= 9.8, H-11), 5.72 (d, 1H, J= 9.8, H-10), 4.66 (s, 2H, H-15), 3.76 (s, 3H, -OCH_3), 2.73 (m, 2H, H-7), 2.29 (s, 3H, H-14), 2.25 (s, 3H, H-13), 1.70 (m, 2H, H-8), 1.41 (s, 3H, H-12); **^{13}C-NMR** (methanol-d_4) δ 157.2 (s, C-3), 152.2 (s, C-2'), 148.5 (s, C-5'), 138.0 (s, C-1)*, 137.9 (s, C-5)*, 132.4 (s, C-6), 131.5 (d, C-10), 126.0 (s, C-2), 124.3 (d, C-11), 123.2 (s, C-1'), 117.5 (d, C-3'), 116.4 (d, C-4'), 113.8 (d, C-6'), 111.7 (d, C-4), 79.0 (s, C-9), 56.3 (t, C-15), 56.0 (q, -OCH_3), 41.2 (t, C-8), 26.2 (q, C-12), 24.8 (t, C-7), 20.6 (q, C-13), 15.2 (q, C-14); **EIMS** (70 eV) *m/z* 354 (2), 336 (12), 179 (10), 162 (23), 161 (100); **HREIMS** Obsd. *m/z*= 354.1853 $(M)^+$, $C_{22}H_{26}O_4$ requires *m/z*= 354.1831.

Renierin A (7): Yellowish oil; $[\alpha]_D$= -12.5° (c= 0.2, $CHCl_3$); **IR** (film) 3500-3200, 1690, 1640, 1450 cm^{-1}; **UV** ($CHCl_3$) λ_{max} 245 (ε=7550) nm; **^{1}H-NMR** (methanol-d_4) δ 6.57 (d, 1H,J= 8.7, H-3'), 6.52 (d, 1H, J= 3.0, H-6'), 6.43 (dd, 1H, J= 8.7, 3.0, H-4'), 5.77 (bs, 1H, H-4), 5.38 (bt, 1H, J= 7.3, H-10), 3.24 (d, 2H, J= 7.3, H-11), 2.44 (d, 1H, J= 17.2, H-2), 2.16 (m, 2H, H-8), 1.99 (m, 1H, H-6), 1.98 (bs, 3H, H-13), 1.96 (d, 1H, J= 17.2, H-2), 1.88 (m, 1H, H-7), 1.72 (s, 3H, H-12), 1.50 (m, 1H, H-7), 1.07 (s, 3H, H-14), 0.98 (s, 3H, H-15); **^{13}C-NMR** (methanol-d_4) δ 202.3 (s, C-3), 170.1 (s, C-5), 151.1 (s, C-2'), 149.3 (s, C-5'), 136.2 (s, C-9), 130.0 (s, C-1'), 125.4 (d, C-10), 125.2 (d, C-4), 117.2 (d, C-6'), 116.5 (d, C-3'), 113.9 (d, C-4'), 51.5 (d, C-6), 48.1 (t, C-2), 40.7 (t, C-8), 37.4 (s, C-1), 29.6 (t, C-7), 29.4 (t, C-11), 28,9 (q, C-15), 27.4 (q, C-14), 24.8 (q, C-13),

16.2 (q, C-12); **EIMS** (70 eV) *m/z* 328 (5), 163 (15), 161 (17), 151 (37), 138 (27), 137 (13), 135 (25), 123 (100); **HREIMS** Obsd. *m/z*= 328.2057 $(M)^+$, $C_{21}H_{28}O_3$ requires *m/z*= 328.2038.

Renierin B (8): Yellowish oil; **IR** (film) 3500-3200, 1640, 1450 cm^{-1}; **UV** ($CHCl_3$) λ_{max} 240 (ε=7520) nm; **^{1}H-NMR** (methanol-d_4) δ 6.58 (d, 1H, J= 8.6, H-3'), 6.53 (d, 1H, J= 3.0, H-6'), 6.44 (dd, 1H, J= 8.6, 3.0, H-4'), 6.24 (bs, 1H, H-2), 5.39 (bt, 1H, J= 7.4, H-10), 3.67 (s, 3H, -OCH_3), 3.25 (d, 2H, J= 7.4, H-11), 2.35 (m, 2H, H-7), 2.13 (m, 2H, H-8), 1.97 (s, 3H, H-13), 1.78 (s, 3H, H-12), 1.21 (s, 6H, H-14 and H-15); **^{13}C-NMR** (methanol-d_4) δ 196.2 (s, C-4), 173.3 (s, C-6), 151.8 (s, C-2'), 148.5 (s, C-5'), 144.6 (s, C-3), 137.8 (s, C-9), 134.2 (s, C-5), 131.0 (s, C-1'), 124.4 (d, C-10), 120.8 (d, C-2), 117.1 (d, C-6'), 116.6 (d, C-3'), 113.9 (d , C-4'), 55.7 (q, -OCH_3), 41.1 (s, C-1), 40.3 (t, C-8), 30.0 (t, C-7), 29.2 (t, C-11), 25.3 (q, C-14 and C-15), 16.3 (q, C-12), 13.0 (q, C-13) ; **EIMS** (70 eV) *m/z* 356 (2), 179 (100), 163 (20), 162 (18), 161 (43); **HREIMS** Obsd. *m/z*= 356.2021 $(M)^+$, $C_{22}H_{28}O_4$ requires *m/z*= 356.1988.

Conversion of panicein A hydroquinone (10) into panicein A_2 (3): A solution of panicein A hydroquinone (**10**, 4 mg) in acetone containing K_2CO_3 (2 mg) was refluxed for 1 hour. The suspension was cooled, filtered and the solvent evaporated. The residue was purified by HPLC (hexane-isopropanol, 98:2) to obtain panicein A_2 (**3**, 3 mg).

Reduction of panicein B_3 (12) to panicein G (5): A solution 1M of lithium aluminum hydride in Et_2O (0.2 mL) was added to panicein B_3 (10 mg) in dry Et_2O (2 ml) and the resulting suspension was stirred overnight at room temperature. Excess reagent was destroyed by careful addition of methanol and the mixture was filtered through a small silica gel column using methanol. The residue was purified on a semipreparative TLC plate using EtOAc to obtain panicein G (**5**, 4 mg).

Conversion of panicein F_1 (4) into panicein F_2 (6): Panicein F_1 (**4**, 3mg) was treated with K_2CO_3 in refluxing acetone for 1 h. The suspension was cooled, filtered and the solvent evaporated. The residue was purified by HPLC (hexane-acetate, 8:2) to obtain panicein F_2 (**6**, 2 mg).

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5.- Though the extraction and isolation procedures followed were extremely mild and the presence of the chromenols **3** and **6** was detected by ^{1}H NMR from the first examination, since both compounds show no significant values of optical rotations the possibility that they might be artefacts can not be disregarded.

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