Free-Radical-Scavenging and Xanthine Oxidase Inhibitory Constituents from *Stereospermum personatum*[§]

U. Sampath Kumar,[†] Ashok K. Tiwari,[‡] S. Venkat Reddy,[†] P. Aparna,[†] R. Jagadeeshwar Rao,[†] A. Zehra Ali,[‡] and J. Madhusudana Rao*,[†]

Natural Products Laboratory, Division of Organic Chemistry-I, and Division of Pharmacology, Indian Institute of Chemical Technology, Hyderabad-500 007, India

Received March 10, 2005

Bioassay-guided fractionation of different extracts of both stem and stem bark of *Stereospermum personatum* led to the isolation of free-radical-scavenging and xanthine oxidase inhibitory molecules along with three new anthraquinones, sterequinones F−H (**1**−**3**), a new naphthoquinone, sterequinone I (**4**), two new phenethyl esters, 2(4′-hydroxyphenyl)ethyl undecanoate (**14**) and 2(4′-hydroxyphenyl)ethyl nonacosanoate (**15**), and a new 3,4,5-trimethoxycinnamyl ether, 2-methoxy-4-[3′-(3″,4″,5″trimethoxyphenyl)allyloxymethyl]phenol (**16**), together with known compounds. The antioxidant and xanthine oxidase inhibitory potentials of the isolated compounds are reported.

Stereospermum personatum Hassk-Chatterjii (Bignoniaceae) is an Ayurvedic medicinal plant used in the traditional Indian System of medicine[1−3] and is widely advocated as a diuretic, antiinflammatory and in preparations for hemorrhoids, vesicle calculi and for cardiotonic, diabetic, cancer, renal, and hyperacidity disease conditions.[4] In a continuation of our search to identify potential antioxidants derived from plants[5] we have tested the free-radical-scavenging (DPPH) and xanthine oxidase inhibitory potential of extracts of various parts of *S. personatum*. Although the petroleum ether and $CHCl_3$ extracts of the stem bark displayed mild free-radical-scavenging activity, the petroleum ether extract resulted in the isolation of biogenetically related quinones.[6] The $CHCl_3$ extract of the stem bark furnished three new anthraquinones, sterequinones F−H (**1**−**3**), a new naphthoquinone, sterequinone I (**4**), norviburtinal (**5**) (Figure 1), vanillin, and ferulic acid. The acetone extract displayed potent free-radical-scavenging and moderate xanthine oxidase inhibition and yielded three known iridoid glycosides (**6**−**8**). The $CHCl_3$ extract of the stem also displayed antioxidant and xanthine oxidase inhibitory potential and afforded two new esters (**14**, **15**) of 4-hydroxyphenylethanol, a new 3,4,5-trimethoxycinnamyl ether (**16**) (Figure 1), and several known compounds. The structure of **16** was confirmed by spectroscopic methods and synthesis. Lignans (**25**−**28**) that were isolated in substantial amounts were evaluated for their antioxidant and xanthine oxidase inhibition potentials.

Results and Discussion

Sterequinone F (**1**) was isolated as a pale yellow solid. The molecular formula was deduced from HREIMS analysis [M^+] (*m/z* 292.1088), ^{13}C NMR, and DEPT experiments as $C_{19}H_{16}O_3$. The IR spectrum displayed absorptions at 1716 and 1667 cm^{-1} due to carbonyl functionalities. The ^1H NMR spectrum (Table 1) revealed two two-proton multiplets, at δ 8.25 and 7.75, indicating a typical 1,2-disubstituted aromatic ring. Two doublets at δ 8.18 (1H, J = 8.3 Hz) and 7.58 (1H, J = 8.3 Hz) were indicative of two *ortho*-coupled aromatic protons (H-4 and 3). Two two-proton triplets at δ 3.06 and 2.75 accounted for the presence of two adjacent methylene groups (H-11 and 12). Two three-proton singlets at δ 2.78 and 2.18 are due to aromatic and acetyl methyl groups, respectively. The data showed resemblance to those of anthrakunthone isolated from *Stereospermum kunthianum*[7] with a different aromatic substitution. The ^{13}C NMR data (Table 1) indicated the presence of 19 carbons, which were sorted by DEPT into two methyls, two methylenes, six methines, and nine quaternary carbons. The above data led to the structure **1**, named sterequinone F, a new 7-deoxy analogue of anthrakunthone.

Sterequinone G (**2**) was isolated as a pale yellow semisolid. Its molecular formula was deduced as $C_{20}H_{18}O_4$ by HREIMS [M^+] (*m/z* 322.1196), which was consistent with ^{13}C NMR and DEPT data. Its IR spectrum displayed carbonyl bands at 1713 and 1665 cm^{-1}. The ^1H NMR spectrum (Table 1) displayed features similar to those of stereqinone-F (**1**) but with a different aromatic substitution pattern. Two one-proton doublets at δ 8.20 (J = 8.5 Hz) and 7.68 (J = 2.5 Hz) and a one-proton double doublet at δ 7.22 (J = 8.5, 2.5 Hz) suggested the presence of a 1,2,4-trisubstituted aromatic ring. Comparison of ^1H, ^{13}C NMR and MS data with those of **1** revealed that **2** had an aromatic *O*-methyl group at δ 3.98 with a corresponding signal at δ 55.8 in its ^{13}C NMR spectrum (Table 1). The position of the *O*-methyl group was established by HMBC correlations (Figure 2) of C-10a with H-8 and H-6 and of C-8a with H-5. The above data showed that stereqinone G had a structure as presented in **2**, a new 7-methoxy analogue of anthrakunthone.

Sterequinone H (**3**) was isolated as a pale yellow solid. It had a molecular formula of $C_{19}H_{18}O_3$ by the analysis of its HREIMS [M^+] (*m/z* 294.1246). The IR spectrum showed absorption bands at 1668 and 1651 cm^{-1} assignable to two carbonyl groups. The ^1H NMR spectrum (Table 1) indicated the presence of aromatic substitution identical with that in stereqinone F (**1**) but with a different side chain. A one-proton multiplet at δ 3.85 was typical of an aliphatic hydroxy methine (H-13). Two multiplets (2H) at δ 2.90 and 1.75 suggested the presence of two adjacent methylene groups, the former being characteristic of a benzylic methylene. A three-proton doublet at δ 1.25 (J = 6.0 Hz)

[§] IICT Communication No. 050613.
* Corresponding author. Tel: +91-40-2719 3166. Fax: +91-40-2716 0512. E-mail: janaswamy@iict.res.in.
[†] Natural Products Laboratory, Division of Organic Chemistry-I.
[‡] Division of Pharmacology.

10.1021/np058036y CCC: $30.25 © 2005 American Chemical Society and American Society of Pharmacognosy
Published on Web 10/29/2005

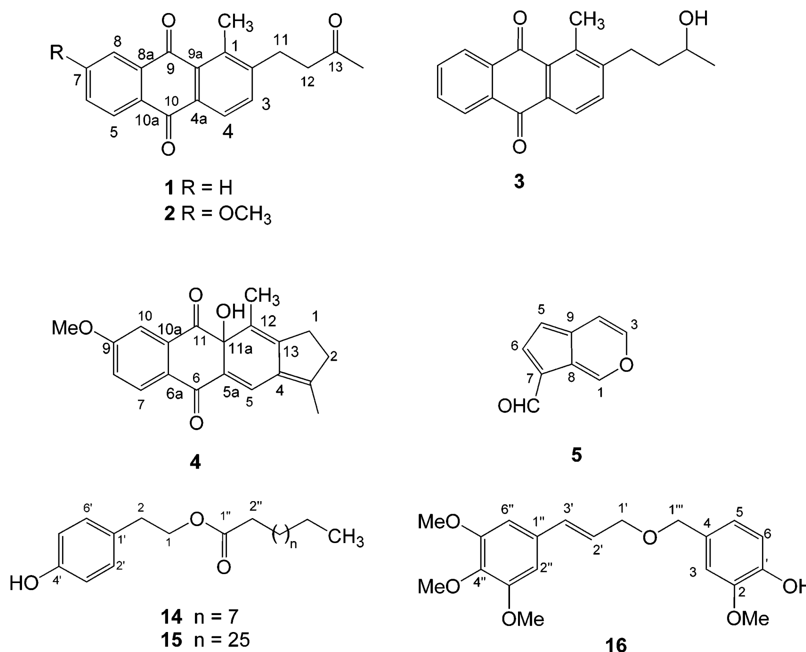
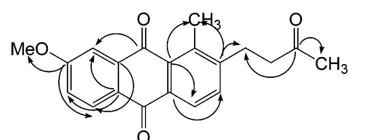


Figure 1.

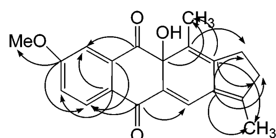
Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**4** in CDCl$_3$[a]

position	**1** δ_H	**1** δ_C	**2** δ_H	**2** δ_C	**3** δ_H	**3** δ_C
1		139.8 C		139.8 C		139.9 C
2		147.7 C		147.2 C		149.0 C
3	7.58 d (8.3)	134.0 CH	7.55 d (8.0)	134.4 CH	7.55 d (8.5)	134.0 CH
4	8.18 d (8.3)	125.6 CH	8.18 d (8.0)	125.6 CH	8.15 d (8.5)	125.5 CH
4a		135.2 C		134.1 C		135.3 C
5	8.25 m	126.4 CH	8.20 d (8.5)	129.0 CH	8.25 m	126.4 CH
6	7.75 m	133.3 CH	7.22 dd (8.5, 2.5)	120.6 CH	7.75 m	133.3 CH
7	7.75 m	134.3 CH		164.3 C	7.75 m	134.4 CH
8	8.25 m	127.2 CH	7.68 d (2.5)	109.9 CH	8.25 m	127.2 CH
8a		133.5 C		137.3 C		133.1 C
9		185.6 C		189.0 C		185.9 C
9a		132.0 C		133.7 C		132.1 C
10a		132.6 C		126.3 C		132.7 C
10		183.5 C		182.3 C		183.6 C
11	3.06 t (7.2)	29.6 CH$_2$	3.10 t (7.2)	28.1 CH$_2$	2.90 m	29.6 CH$_2$
12	2.75 t (7.2)	43.3 CH$_2$	2.78 t (7.2)	43.4 CH$_2$	1.75 m	39.3 CH$_2$
13		206.8 C		206.9 C	3.85 m	67.5 CH
1-CH$_3$	2.78 s	17.4 CH$_3$	2.75 s	17.4 C	2.80 s	17.4 CH$_3$
13-CH$_3$	2.18 s	30.0 CH$_3$	2.20 s	29.3 CH$_3$	1.25 d	23.8 CH$_3$
OCH$_3$			3.98 s	55.8 CH$_3$		

[a] ^{1}H and ^{13}C NMR spectra recorded for **1** and **3** at 200 and 50 MHz, respectively, for **2** at 300 and 75.462 MHz, respectively. Chemical shifts presented in ppm (δ) with TMS as internal standard; J values are given in Hz in parentheses.



HMBC correlations of sterequinone-G (**2**)



HMBC correlations of sterequinone-I (**4**)

Figure 2.

accounted for the terminal methyl group. The ^{13}C NMR spectrum (Table 1) indicated 19 carbons. The presence of the carbinol function was evident by the presence of a methine carbon at δ 67.5. The above data were consistent with structure **3** (Figure 1) for sterequinone H.

Sterequinone I (**4**) was isolated as a pale yellow solid. The molecular formula was determined as $C_{20}H_{18}O_4$ by HRFABMS $[M + H]^+$ (m/z 323.1296), which was consistent with ^{13}C NMR and DEPT data. The IR spectrum indicated two sharp bands at 1717 and 1664 cm^{-1} accounting for two carbonyl groups. The ^{1}H NMR spectrum of compound **4** showed two one-proton doublets at δ 8.18 ($J = 8.6$ Hz) and 7.60 ($J = 2.6$ Hz) and a one-proton double doublet at δ 7.20 ($J = 8.6$, 2.6 Hz), suggesting a typical 1,2,4-trisubstituted aromatic ring. A sharp singlet at δ 3.98 (3H) accounted for an aromatic O-methyl group. A double multiplet at δ 3.02 (2H) and a multiplet at δ 2.25 (2H) indicated the presence of two adjacent methylene groups (H-2 and H-1). Two singlets at δ 2.70 (3H) and 1.65 (3H) were indicative of two olefinic methyl groups. The downfield shift of the former could be due to its proximity to both the carbonyl and

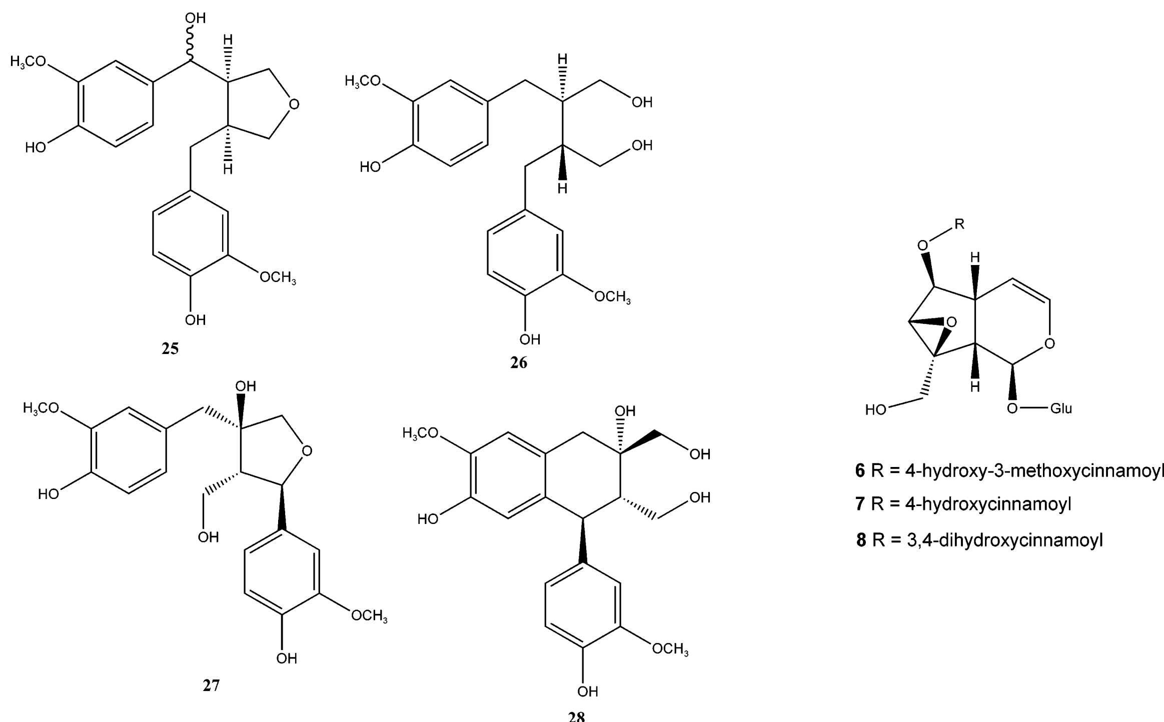


Figure 3.

hydroxyl groups. The above ^{1}H NMR data showed resemblance to those of kigelinol isolated from *Kigelia pinnata*.[8] The ^{13}C NMR spectrum revealed the presence of 20 carbons. The position of the aromatic *O*-methyl group was established by HMBC correlations (Figure 2), where C-6a showed correlations with H-10 and H-8. C-10a and C-9 showed correlations with H-7. The above data established the structure **4** for sterequinone I. Sterequinone I probably represents the missing link in the biogenetic sequence of sterequinone B to sterequinone D previously isolated by us.[6]

Norviburtinal (**5**) was isolated as an orange powder and was identified by comparing its spectroscopic data with literature data.[9] However, the ^{13}C NMR data whose assignments are based on a DEPT experiment and in comparison with halitunal[10] are reported here for the first time.

The acetone extract of the stem bark upon further purification yielded substantial amounts of three iridoid glycosides (Figure 3), which were identified as (−)-6-feruloyl catalpol (**6**),[11] (−)-specioside (**7**),[12] and (−)-verminoside (**8**)[13] by comparison of spectroscopic data with literature values.

The powdered stem of *S. personatum* was first defatted with petroleum ether followed by the extraction with $CHCl_3$. Further purification of the $CHCl_3$ extract yielded three new compounds (**14**−**16**) (Figure 1) along with several known compounds. The known compounds were identified as lapachol (**9**),[14] dehydro-α-lapachone (**10**),[14] coniferaldehyde (**11**),[15] sinapaldehyde (**12**),[16] 3,4,5-trimethoxycinnamaldehyde (**13**),[17] pinoresinol (**24**),[18] 7′-hydroxydivanillyltetrahydrofuran (**25**),[19] (−)-secoisolariciresinol (**26**),[20] (−)-olivil (**27**),[21] and (+)-cycloolivil (**28**)[21] (Figure 3). Except lapachol, all the remaining compounds were isolated for the first time from this plant.

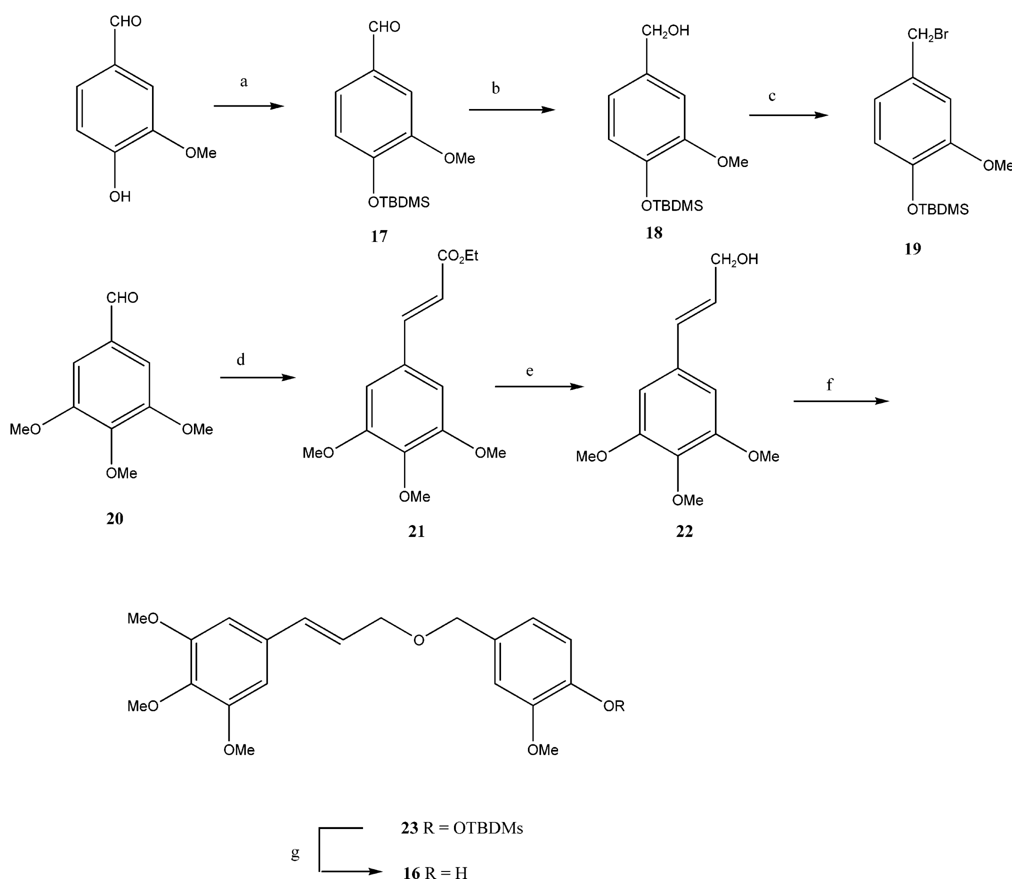
Compound **14** was obtained as a white solid. The molecular formula was deduced from its positive ion FABMS as $C_{19}H_{30}O_3$, which showed an $[M + Na]^+$ ion at *m/z* 329. The IR spectrum revealed the presence of a hydroxyl group at 3408 cm^{-1} and an ester carbonyl function at 1733 cm^{-1}. The ^{1}H NMR spectrum of compound **14** showed two two-proton doublets at δ 7.05 (2H, $J = 9.0$ Hz) and 6.72 (2H, $J = 9.0$ Hz), suggesting the presence of a 1,4-disubstituted aromatic ring. Two triplets were observed at δ 2.85 (2H) and 4.22 (2H), the former attributed to a benzylic methylene and the latter to an oxymethylene group of an ester ($-CH_2-O-$). A broad singlet at δ 4.60, which was exchanged with D_2O, accounted for a phenolic hydroxyl group. The above data suggested the presence of a 4-hydroxyphenylethyl group, which was substantiated by a fragment at *m/z* 121 in its EI MS spectrum.

The ^{1}H NMR spectrum further displayed a three-proton triplet at δ 0.90, which was characteristic of a terminal methyl group of an aliphatic chain. Another triplet at δ 2.30 (2H) was characteristic of a methylene adjacent to a carbonyl group. A broad multiplet at δ 1.30 integrating for 14 protons accounted for the presence of an aliphatic chain comprising seven methylenes. ^{13}C assignments were made by comparing the data with that of bongardol (octacosanoic acid ester of 4-hydroxyphenylethanol) isolated from *Bongardia chrysogonum*.[22] The above data suggested that compound **14** is a new undecanoic acid ester of 4-hydroxyphenylethanol.

Compound **15** was also isolated as a white solid. The molecular formula $C_{37}H_{66}O_3$ was deduced from its FABMS (M^+ 558). The ^{1}H NMR has indicated features similar to compound **14** except for the multiplet at δ 1.30 integrating for 50 protons that indicated the presence of 25 methylenes. These data along with the established molecular mass allowed the structure of compound **15** to be defined as a new nonacosanoate ester of 4-hydroxyphenylethanol.

Compound **16** was isolated as a pale brown semisolid. The molecular formula $C_{20}H_{24}O_6$ was deduced from its HREIMS analysis (*m/z* 360.1562), ^{13}C NMR, and a DEPT experiment. The IR spectrum indicated the presence of a hydroxyl group at 3416 cm^{-1} and an aliphatic ether at 1034 cm^{-1}. The ^{1}H NMR spectrum showed a double triplet at δ 6.25 (1H) and a doublet at δ 6.50 (1H, $J = 15.0$ Hz) indicative of a *trans* double bond. A sharp singlet at δ 6.60 (2H) indicated the presence of two equivalent aromatic

Scheme 1[a]



[a] Reagents and conditions: (a) TBDMSCl, Et_3N, DCM, rt, 8 h; (b) $NaBH_4$, MeOH, rt, 5 h; (c) PBr_3, DCM, −10 °C, 30 min; (d) carbethoxymethylene triphenyl phosphorane, benzene, reflux, 12 h; (e) DIBAL-H, DCM, −10 °C 1 h; (f) sodium hydride, THF, compound **19**, 24 h; (f) TBAF, THF, 10 min.

protons. A two-proton doublet at δ 4.30 ($J = 5.0$ Hz) was indicative of the oxymethylene of an ether function. Its deshielding may be attributed to its allylic nature. Two singlets integrating for six and three protons respectively resonated at δ 3.88 and 3.82, respectively, and suggested the presence of three aromatic *O*-methyl groups. The ^1H NMR spectrum displayed a three-proton singlet at δ 3.92 accounting for one more aromatic *O*-methyl group. A broad singlet at δ 5.52, which was exchanged with D_2O, suggested the presence of a phenolic hydroxyl group. A singlet at δ 4.60 (2H) was characteristic of the benzylic methylene group adjacent to an ether functionality. A doublet at δ 6.85 (2H, $J = 2.5$ Hz) and a singlet at δ 6.90 (1H) indicated the presence of three more aromatic protons. The above data suggested the presence of a 4-hydroxy-3-methoxybenzyl group, which was confirmed by the presence of a fragment at *m/z* 136 in its EIMS spectrum. The ^{13}C NMR spectrum of the compound indicated the presence of 20 carbons, which are consistent with the proposed structure. The above data suggested the structure of compound **16** as shown (Figure 1). The structure was further confirmed via synthesis from vanillin (Scheme 1).

Vanillin was protected as TBDMS ether (**17**) followed by reduction of the aldehyde group of **17** with sodium borohydride to afford 4-*tert*-butyldimethylsilyloxyvanillyl alcohol (**18**) in quantitative yield. The resultant alcohol was converted into the corresponding benzyl bromide (**19**) using phosphorus tribromide under anhydrous conditions.[23] Ethyl 3′,4′,5′-trimethoxycinnamate (**21**) was prepared by the Wittig reaction of 3,4,5-trimethoxybenzaldehyde with carbethoxymethylene triphenylphosphorane in anhydrous benzene. The cinnamic ester was reduced with DIBAL-H to afford the corresponding cinnamyl alcohol (**22**). Alkylation of the cinnamyl alcohol with the benzyl bromide (**19**) using sodium hydride followed by deprotection of the silyl group using tetrabutylammonium fluoride yielded compound **16** with spectroscopic data identical with the natural product.

Iridoids are distributed widely in plants belonging to the Bignoniaceae family[24] and are well known for their broad spectrum of biological properties.[25] Some of the iridoid glycosides are well documented for their free-radical-scavenging and xanthine oxidase inhibitory properties.[26,27] In our search for potential antioxidants derived from plants[5] we have screened the three iridoids (**6**−**8**) for their antioxidant (DPPH) activity as well as potential for xanthine oxidase inhibition. Table 2 summarizes the SC_{50} for free-radical DPPH scavenging and IC_{50} for xanthine oxidase inhibitory concentration of extract as well as compounds isolated from it. Among the three iridoids (−)-verminoside (**8**) displayed strong free-radical-scavenging properties, whereas (−)-specioside (**7**) displayed mild activity. The xanthine oxidase inhibition pattern of these three compounds was in the reverse order with (−)-specioside (**7**) presenting high inhibition followed by (−)-6-feruloylcatalpol (**6**). (−)-Verminoside (**8**) did not display xanthine oxidase inhibitory property.

Lignans possess a broad spectrum of biological activities.[28] Since the lignans 7′-hydroxydivanillyltetrahydrofuran (**25**), (−)-secoisolariciresinol (**26**), (−)-olivil (**27**), and (+)-cycloolivil (**28**) (Figure 3) were obtained in substantial quantities, they were also evaluated for their free-radical-scavenging (DPPH) and xanthine oxidase inhibitory activities. Table 3 displays the free-radical (DPPH)-scavenging and xanthine oxidase inhibitory concentration of extract as well as lignans isolated from it. These compounds

Table 2. Antioxidant and Xanthine Oxidase Inhibitory Activity of Extracts and Isolated Compounds from the Stem Bark of *S. personatum*[a]

compound	antioxidant (DPPH) activity (SC_{50})	xanthine oxidase inhibition (IC_{50})
$CHCl_3$ extract	12.14 μg/mL	NA
sterequinone A-I	NA	NA
ferulic acid	32.51 μM	NA
acetone extract	6.78 μg/mL	54.2 μg/mL
6-feruloylcatalpol (**6**)	40.49 μM	150.4 μM
specioside (**7**)	160.85 μM	79.88 μM
verminocide (**8**)	7.88 μM	NA
allopurinol		34.91 μM
trolox	15.94 μM	

[a] NA, compounds displaying less than 10% activity at 50 μg/mL concentration were not followed for IC_{50} or SC_{50} analysis. IC_{50} or SC_{50} values were determined by linear regression analysis using at least five different concentrations in triplicate and represent the mean of the experiment. SDM were within 10% in any case. IC_{50} are concentrations of compounds or mixtures required to inhibit 50% activity of xanthine oxidase enzyme, and SC_{50}, the concetration of extracts or compounds to scavenge 50% of the free-radical (DPPH) under experimental conditions.

Table 3. Antioxidant and Xanthine Oxidase Inhibitory Activity of Extract and Isolated Compounds from the Stem of *S. personatum*[a]

compound	antioxidant (DPPH) activity (SC_{50})	xanthine oxidase inhibition (IC_{50})
$CHCl_3$ extract	7.47 μg/mL	59.83 μg/mL
compound (**16**)	7.58 μM	NA
secoisolariciresinol (**26**)	12.5 μM	213 μM
cycloolivil (**28**)	17.38 μM	101.38 μM
7′-hydroxydivanillyl-tetrahydrofuran (**25**)	15.16 μM	110.88 μM
olivil (**27**)	15.10 μM	23.63% at 132 μM
trolox	15. 94 μM	
allopurinol		34.91 μM

[a] NA, compounds displaying less than 10% activity at 50 μg/mL concentration were not followed for IC_{50} or SC_{50} analysis. IC_{50} or SC_{50} values were determined by linear regression analysis using at least five different concentrations in triplicate and represent the mean of the experiment. SDM were within 10% in any case. IC_{50} are concentrations of compounds or mixtures required to inhibit 50% activity of xanthine oxidase enzyme, and SC_{50}, the concetration of extracts or compounds to scavenge 50% of the free-radical (DPPH) under experimental conditions.

displayed moderate xanthine inhibitory activity. As far as their xanthine oxidase inhibitory potential is concerned, (+)-cycloolivil (**28**) and 7′-hydroxydivanillyltetrahydrofuran (**25**) were more potent than (−)-secoisolariciresinol (**26**) and (−)-olivil (**27**). While the DPPH free-radical-scavenging activities of (−)-olivil and (+)-cycloolivil are known,[29] the DPPH-scavenging activities for 7′-hydroxydivanillytetrahydrofuran (**25**) and secoisolarciresinol (**26**) are reported here for the first time. Similarly the xanthine oxidase inhibitions of these lignans are also reported for the first time.

The combined application of free-radical scavengers of varied nature with multiple activities is increasingly being advocated for the prevention and treatment of oxidative stress related disorders.[30,31] The presence in substantial yield of free-radical-scavenging, xanthine oxidase inhibitory iridoids and lignans in *S. persontum*, therefore, may provide scientific basis for its use in Ayurvedic preparations prescribed under a variety of disease conditions.

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra were obtained on Varian 200 MHz and Bruker 300 MHz spectrometers using TMS as internal standard. IR spectra were recorded on a Nicolet-740 spectrometer. EIMS was recorded on a VG 70-70 micromass instrument. FABMS and HREIMS were recorded on a VG Auto Spec-M instrument. Column chromatography was performed on silica gel (60−120 mesh). Solvents and reagents were purified according to standard procedures.

Plant Material. Plant material was collected from the forests of Tirumala, Andhra Pradesh, India, in January 2000. It was identified by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati. A voucher specimen of the plant is deposited in Herbarium, Department of Botany, with an accession number 547.

Extraction and Isolation. The shade-dried stem bark of *S. personatum* (2 kg) was powdered and extracted with petroleum ether in a Soxhlet apparatus for 20 h. The material was then extracted with $CHCl_3$ for 20 h followed by extraction with acetone. The $CHCl_3$ extract was evaporated under reduced pressure to obtain a residue (4.0 g). The residue was adsorbed on silica gel and subjected to column chromatography eluted with petroleum ether and by mixtures containing increasing amounts of EtOAc. The fractions eluted at 5, 10, 15, and 20% EtOAc in petroleum ether were collected separately, concentrated, and rechromatographed using silica gel (60−120 mesh) to obtain compounds **1** (20 mg), **2** (8 mg), **3** (20 mg), **4** (12 mg), and **5** (25 mg) in pure form.

The acetone extract (20 g) was purified using column chromatography (silica gel, 60−120 mesh) with $CHCl_3$ and increasing amounts of MeOH. The fractions eluted with 9, 10, and 11% MeOH were collected separately and concentrated to obtain compounds **6** (2.5 g), **7** (5.5 g), and **8** (2.0 g) in pure form.

Similarly, the shade-dried stem of *S. personatum* (2.5 kg) was extracted with petroleum ether in a Soxhlet apparatus followed by the extraction with $CHCl_3$ for 20 h. The $CHCl_3$ extract on evaporation yielded a residue of 22 g. The residue was adsorbed on silica gel (60−120 mesh) and subjected to chromatography by eluting first with $CHCl_3$, followed by $CHCl_3$/acetone (9:1, 8.5:1.5) (I and II) and $CHCl_3$/MeOH (9.5:0.5) (III). Fraction I eluted at 10% acetone was subjected to chromatography over silica gel using $CHCl_3$ with increasing percentage of acetone. The fractions eluted at 2%, 4%, 6%, 8%, and 10% acetone yielded compounds **9** (65 mg), **10** (20 mg), **11** (8 mg), **12** (6 mg), and **13** (8 mg) in pure form. Fraction II eluted at 15% acetone in $CHCl_3$ was also subjected to column chromatography over silica gel with $CHCl_3$ with increasing percentages of acetone. The fractions eluted at 5%, 10%, 15%, and 20% acetone yielded compounds **14** (18 mg), **15** (20 mg), **16** (15 mg), and **24** (6 mg) in pure form. Fraction III was subjected to column chromatography using $CHCl_3$ and MeOH. The fractions eluted at 2%, 3%, 4%, and 5% MeOH in $CHCl_3$ yielded compounds **25** (50 mg), **26** (1 g), **27** (2.5 g), and **28** (2.2 g) in pure form.

Sterequinone F (1): pale yellow solid; UV (MeOH) λ_{max} (log ϵ) 259 (3.45), 252 (3.41) nm; IR(KBr) ν_{max} 2916, 1716, 1667, 1330, 1287, 1169, 760 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* (rel int) 292 (4) [M$^+$], 274 (100), 259 (46), 249 (30), 178 (27), 149 (18), 129(7), 97 (30); HREIMS *m/z* 292.1088 (calcd for $C_{19}H_{16}O_3$, 292.1099).

Sterequinone G (2): pale yellow semisolid; UV (MeOH) λ_{max} (log ϵ) 267 (3.6) nm; IR (KBr) ν_{max} 2918, 2818, 1713, 1665, 1596,1282 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* (rel int) 322 (M$^+$) (10), 304 (85), 225 (35), 165 (30), 149 (50), 81 (65), 57 (100); HREIMS *m/z* 322.1196 (calcd for $C_{20}H_{18}O_4$, 322.1205).

Sterequinone H (3): yellow solid; UV (MeOH) λ_{max} (log ϵ) 278 (3.50), 259 (3.9) nm; IR (KBr) ν_{max} 3456, 2822, 1668, 1651, 1581, 1321, 1127, 759 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* (rel int) 294 [M$^+$] (5), 279 (10), 199 (12), 125 (30), 106 (100); HREIMS *m/z* 294.1246 (calcd for $C_{19}H_{18}O_3$, 294.1255).

Sterequinone I (4): pale yellow solid; UV (MeOH) λ_{max} (log ϵ) 272 (3.92), 252 (3.78) nm; IR (KBr) ν_{max} 3431, 2918, 1717, 1664, 1595, 1272, 1178 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (1H, d, J = 8.6 Hz, H-7), 8.13 (1H, s, H-5), 7.60 (1H, d, J

= 2.6 Hz, H-10), 7.20 (1H, dd, J = 8.6, 2.6 Hz, H-8), 3.98 (3H, s, OCH_3), 3.02 (2H, dm, H-2), 2.70 (3H, s, 12-CH_3), 2.25 (2H, m, H-1), 1.65 (3H, s, 3-CH_3); ^{13}C NMR (75.462 MHz) δ 182.8 (C, C-11), 181.7 (C, C-6), 162.0 (C, C-9), 151.2 (C, C-5a), 148.4 (C, C-4), 138.8 (C, C-10a), 133.2 (C, C-13), 132.9 (C, C-3), 129.4 (C, C-6a), 127.9 (CH, C-7), 126.8 (C, C-12), 119.6 (CH, C-8), 118.2 (CH, C-5), 107.3 (CH, C-10), 79.6 (C, C-11a), 54.2 (CH_3, OCH_3), 39.4 (CH_2, C-1), 28.0 (CH_2, C-2), 27.8 (CH_3, 3-CH_3), 17.0 (CH_3, 12-CH_3); HRFABMS m/z 323.1296 (calcd for $C_{20}H_{19}O_4$, 323.1283).

Norviburtinal (5): orange powder; IR (KBr) ν_{max} 1645, 1559, 1460, 1396, 1117, 1023, 965, 736 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.85 (1H, s, CHO), 9.21 (1H, s, H-1), 7.82 (1H, d, J = 3.5 Hz, H-3), 7.79 (1H, d, J = 6.0 Hz, H-6), 7.30 (1H, d, J = 6.0 Hz, H-5), 6.59 (1H, d, J = 3.5 Hz, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ 184.7 (CH, CHO), 151.1 (CH, C-1), 146.1 (CH, C-6), 143.2 (CH, C-3), 134.4 (C, C-9), 124.2 (C, C-8), 123.1 (C, C-7), 110.5 (CH, C-5), 111.1 (CH, C-4); EIMS m/z (rel int) 146 [M$^+$] (78), 145 (100), 117 (30), 89 (37.5), 63 (43.7), 43 (31.2).

2-(4′-Hydroxyphenyl)ethyl undecanoate (14): white solid; UV (MeOH) λ_{max} (log ϵ) 277 (3.2) nm; IR (KBr) ν_{max} 3408, 2918, 1733, 1517, 1464, 1175 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.05 (2H, d, J = 9.0 Hz, H-2′ and 6′), 6.72 (2H, d, J = 9.0 Hz, H-3′ and 5′), 4.60 (brs, ArOH), 4.22 (2H, t, J = 6.9 Hz, H-1), 2.85 (2H, t, J = 6.9 Hz, H-2), 2.30 (2H, t, J = 6.9 Hz, H-2″), 1.60 (2H, m, H-3″), 1.30 (14H, s, H-4″ to H-10″), 0.90 (3H, t, J = 6.2 Hz, H-11″); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.8 (C, C-1″), 154.4 (C, C-4′), 129.9 (CH, C-6′ and 2′), 129.8 (C, C-1′), 115.3 (CH, C-3′ and 5′), 64.9 (CH_2, C-1), 34.3 (CH_2, C-2), 31.9 (CH_2, C-2″), 29.7−22.7 (CH_2, C-3″-10″), 14.1 (CH_3, C-11″); FABMS m/z 329 [M + Na]$^+$.

2-(4′-Hydroxyphenyl)ethyl nonacosanoate (15): colorless solid; UV (MeOH) λ_{max} (log ϵ) 278 (3.2) nm; IR (KBr) ν_{max} 3406, 2917, 1733, 1517, 1464, 1175 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (2H, d, J = 9.0 Hz, H-2′ and 6′), 6.75 (2H, d, J = 9.0 Hz, H-3′ and 5′), 4.50 (brs, ArOH), 4.25 (2H, t, J = 6.9 Hz, H-1), 2.85 (2H, t, J = 6.9 Hz, H-2), 2.30 (2H, t, J = 6.9 Hz, H-2″), 1.60 (2H, m, H-3″), 1.30 (50H, s, H-4″ to H-28″), 0.92 (3H, t, J = 6.2 Hz, H-29″); ^{13}C NMR (75.462 MHz, $CDCl_3$) δ 173.9 (C, C-1″), 154.3 (C, C-4′), 129.9 (CH, C-2′ and 6′), 129.8 (C, C-1′), 115.3 (CH, C-3′ and 5′), 64.9 (CH_2, C-1), 34.3 (CH_2, C-2), 31.9 (CH_2, C-2″), 29.6−22.6 (CH_2, C-3″−28″), 14.0 (CH_3, C-29″); FABMS m/z 558.

2-Methoxy-4-[3′-(3″,4″,5″-trimethoxyphenyl)allyloxymethyl]phenol (16): pale brown semisolid; UV (MeOH) λ_{max} (log ϵ) 285 (2.92) nm; IR (KBr) ν_{max} 3416, 1514, 1480, 1275, 1239, 1125, 1034, 757 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.90 (1H, s, H-3), 6.85 (2H, d, J = 2.5 Hz, H-5 and 6), 6.60 (2H, s, H-2″ and 6″), 6.50 (1H, d, J = 15.0 Hz, H-3′), 6.25 (1H, dt, J = 15, 5.0 Hz, H-2′), 5.52 (brs, ArOH), 4.60 (2H, s, H-1‴), 4.30 (2H, d, J = 5.0 Hz, H-1′), 3.92 (3H, s, 2-OCH_3), 3.88 (6H, s, 3″ and 5″-OCH_3), 3.82 (3H, s, 4″-OCH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 153.3 (C, C-3″ and 5″), 146.5 (C, C-2), 143.8 (C, C-1), 138.2 (C, C-4″), 133.0 (C, C-4), 132.4 (C, C-1″), 131.1 (CH, C-3′), 128.0 (CH, C-2′), 120.2 (CH, C-5), 114.3 (CH, C-3), 110.0 (CH, C-6), 103.8 (CH, C-2″ and 6″), 65.3 (CH_2, C-1‴), 63.5 (CH_2, C-1′), 60.8 (CH_3, 4″-OCH_3), 56.1 (CH_3, 3″ and 5″-OCH_3), 55.9 (CH_3, 2-OCH_3); EIMS 360 (M$^+$) (28), 193 (12), 136 (100), 82 (42); HREIMS m/z 360.1562 (calcd for $C_{20}H_{24}O_6$, m/z 360.1572).

Synthesis of Compound 16 (Scheme 1). 4-*tert*-Butyldimethylsilyloxy-3-methoxybenzaldehyde (17). Vanillin (1 g, 0.0065 mol) was dissolved in anhydrous CH_2Cl_2 (20 mL) and Et_3N (0.796 g, 0.0078 mole). The mixture was cooled to 0 °C. To this solution was added slowly t-BDMSiCl (0.99 g, 0.0065 mole), and the mixture was stirred under nitrogen for 8 h. After completion of the reaction the reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with H_2O (50 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give **17** (1.50 g, 86%) as a liquid: IR (KBr) ν_{max} 2931, 1686, 1508, 900, 782 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.80 (1H, s, CHO), 7.35 (2H, m, H-2 and 6), 6.92 (1H, d, J = 9.1 Hz, H-3), 3.90 (3H, s, OCH_3), 1.05 (9H, s, $(CH_3)_3$-C), 0.20 (6H, s, Si-$(CH_3)_2$); EIMS m/z 264 (M$^+$).

(b) 4-*tert*-Butyldimethylsilyloxy-3-methoxybenzyl alcohol (18). To a solution of **17** (1.32 g, 0.005 mol) in MeOH (15 mL) was added $NaBH_4$ (0.141 g, 0.003 mol) slowly at 0 °C, and the mixture was stirred at room temperature for 5 h. The solvent was evaporated under vacuum. The residue was dissolved in EtOAc (50 mL), washed with H_2O (50 mL), dried over anhydrous Na_2SO_4, and concentrated to obtain **18** (1.2 g, 90%) as a colorless liquid: IR (KBr) ν_{max} 2935, 1514, 1465, 1293, 899 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.85 (1H, s, H-2), 6.75 (2H, s, H-5 and 6), 4.58 (2H, Ar-CH_2), 3.80 (3H, OCH_3), 1.40 (brs, OH), 1.0 (9H, $(CH_3)_3$-C), 0.18 (6H, Si-$(CH_3)_2$); EIMS m/z 210 (M$^+$ − $(CH_3)_3$C).

(c) 4-*tert*-Butyldimethylsilyloxy-3-methoxybenzyl bromide (19). To a solution of **18** (0.350 g, 0.001 mol) in anhydrous CH_2Cl_2 (5 mL) was added slowly PBr_3 (0.11 g, 0.042 mol) in anhydrous CH_2Cl_2 (1 mL) at −10 °C under N_2 atmosphere, and the mixture was stirred for 30 min. Solid $NaHCO_3$ (0.100 g) was added, and the organic layer was decanted and washed with CH_2Cl_2 again. The combined organic layers are concentrated to obtain **19** (0.380 g, 87%) as a colorless liquid. The product was immediately used for the subsequent reaction as it decomposed rapidly. ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.70−6.82 (3H, m, H-2, 5, and 6), 4.42 (2H, Ar-CH_2), 3.80 (3H, s, OCH_3), 1.0 (9H, $(CH_3)_3$-C), 0.18 (6H, Si-$(CH_3)_2$).

(d) Ethyl 3′,4′,5′-trimethoxycinnamate (21). 3,4,5-Trimethoxybenzaldehyde (0.49 g, 0.002 mol) and carbethoxymethylenetriphenyl phosphorane (0.869 g, 0.002 mol) were dissolved in anhydrous benzene (15 mL). The mixture was refluxed under nitrogen for 12 h, the solvent was removed under vacuum, and the product was purified by column chromatography (silica gel, 60−120 mesh). The fractions eluted at 20% EtoAc in petroleum ether yielded **21** (0.55 g, 83%): IR (KBr) ν_{max} 2932, 1710, 1512, 1467, 1274, 1160 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.58 (1H, d, J = 17.3 Hz, H-β), 6.70 (2H, s, H-2′ and 6′), 6.30 (1H, d, J = 17.3 Hz, H-α), 4.20 (2H, q, H-2), 3.88 (6H, s, 3′-OCH_3 and 5′-OCH_3), 3.82 (3H, s, 4′-OCH_3), 1.30 (3H, t, H-3); EIMS m/z 266 (M$^+$).

(e) 3′,4′,5′-Trimethoxycinnamyl alcohol (22). Ethyl 3′,4′,5′,-trimethoxycinnamate **21** (0.266 g, 0.001mol) was dissolved in anhydrous CH_2Cl_2 (10 mL), and the mixture was cooled to −15 °C. To this mixture was added slowly 1.18 mL (0.355 g, 0.002 mol) of a 30% solution of DIBAL-H in hexane. The mixture was stirred for 1 h under nitrogen atmosphere. MeOH (0.5 mL) was added slowly followed by the addition of a saturated solution of sodium potassium tartarate (15 mL). The mixture was stirred vigorously for 1 h, and the layers were separated. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to yield **22** (0.180 g, 81%) as a colorless liquid: IR (KBr) ν_{max} 3400, 1580, 1450 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.82 (2H, s, H-2′ and 6′), 6.55 (1H, d, J = 14.4 Hz, H-β), 6.20 (1H, m, H-α), 4.30 (2H, d, J = 6.4 Hz, H-1), 3.88 (6H, s, 3′ and 5′ OCH_3), 3.82 (3H, s, 4′-OCH_3); EIMS m/z 224 (M$^+$).

(f) *tert*-Butyl-{2-methoxy-4-[3′-(3″,4″,5″-trimethoxyphenyl)allyoxymethyl]phenoxy}dimethylsilane (23). To a suspension of NaH (0.029 g, 0.0012 mol) in anhydrous THF (3 mL) was added slowly **22** (0.180 g, 0.0008 mol) in anhydrous THF (5 mL) under nitrogen atmosphere at room temperature, and the mixture was stirred for 0.5 h. To this mixture was added **18** (0.267 g, 0.008 mol) in anhydrous THF (3 mL), and the mixture was stirred for 24 h at room temperature. The reaction mixture was poured into ice and extracted with EtOAc (2 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography over silica gel (60−120 mesh). The fractions eluted at 20% EtOAc in petroleum ether gave **23** (0.050 g): IR (KBr) ν_{max} 2930, 1512, 1465, 1129, 913, 794 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.0 (1H, s, H-2), 6.78 (2H, m, H-5 and 6), 6.55 (2H, s, H-2″ and 6″), 6.52 (1H, d, J = 17.3 Hz, H-3′), 6.20 (1H, dt, H-2′), 4.82 (2H, s, H-1‴), 4.25 (2H, d, J = 4.3 Hz, H-1′), 3.82 (9H, s, 3″, 5″, and 3-OCH_3), 3.80 (3H, s, 4″-OCH_3), 1.0 (9H, s, $(CH_3)_3$-C), 0.20 (6H, s, Si-$(CH_3)_2$); FABMS m/z 475 (M + H)$^+$.

(g) 2-Methoxy-4-[3′-(3″,4″,5″-trimethoxyphenyl)allyloxymethyl]phenol (16). To a solution of compound **23** (0.020 g) in THF (2 mL) was added a 1 M solution of TBAF (0.05 mL) in THF. The reaction was completed immediately, as

evident from TLC (solvent system: petroleum ether/EtOAc, 70:30). To the mixture was added H_2O (5 mL), and the product was extracted with EtOAc to afford the target compound **16** (0.010 g), which was homogeneous by TLC and identical with the natural product in all aspects.

Bioassay Procedures. Determination of free-radical DPPH scavenging and xanthine oxidase activity was done as per the method described earlier.[32]

Acknowledgment. We thank Dr. J. S. Yadav, Director, IICT, for his invaluable suggestions. S.V.R. and R.J.R. thank CSIR and UGC, respectively, for their fellowships.

References and Notes

(1) Sastry, B. N. *Wealth of India. Raw Materials*; CSIR: New Delhi, 1972; Vol 10, pp 49−50.
(2) Bhatnagar S. S.; Santapau, H.; Desa, J. D. H.; Maniar, A. C.; Ghadial, N. C.; Soloman, M. J.; Yellore, S.; Rao, T. N. *Indian J. Med. Res.* **1961**, *49*, 799.
(3) Bakuni, D. S.; Dhar, M. J.; Dhar, M. M.; Dhawan, B. N.; Gupta, B.; Srimal, R. C. *Indian J. Exp. Biol.* **1971**, *9*, 91−102.
(4) Varier, P. S. *Indian Medicinal Plants*; Orient Longman: India, 1994; Vol. 5, pp 193−197.
(5) Tiwari, A. K.; Srinivas, P. V.; Kumar, S. P.; Rao, J. M. *J. Agric. Food Chem.* **2001**, *49*, 4642−4645.
(6) Kumar, U. S.; Aparna, P.; Rao, R. J.; Rao, T. P.; Rao, J. M. *Phytochemistry* **2003**, *63*, 925−929.
(7) Onegi, B.; Kraft, C.; Kohler, I.; Freund, M.; Jenett-Siems, K.; Siems, K.; Beyer, G.; Melzig, M. F.; Bienzle, U.; Eich, E. *Phytochemistry* **2002**, *60*, 39−44.
(8) Akunyili, D. N.; Houghton, P. N. *Phytochemistry* **1993**, *32*, 1015−1018.
(9) Joshi, K. C.; Singh, P.; Taneja, S.; Cox, P. J.; Howie, R. A.; Thomson, R. H. *Tetrahedron* **1982**, *38*, 2703−2708.
(10) Koehn, F. E.; Gunasekera, S. P.; Neil, D. N.; Cross, S. S. *Tetrahedron Lett.* **1991**, *32*, 169−172.
(11) Stuppner, H.; Wagner, H. *Planta Med.* **1989**, *55*, 467−469.
(12) De Silva, L. B.; Herath, W. H. M. M.; Jennings, R. C.; Mahendran, M.; Wanningama, G. P. *Indian J. Chem.* **1982**, *21*B, 703−704.
(13) Sticher, O. *Helv. Chim. Acta* **1979**, *62*, 530−535.
(14) Burnett, A. R.; Thomson, R. H. *J. Chem. Soc. C* **1967**, *1261*, 2100.
(15) Sy, L. K.; Brown, G. D. *Phytochemistry* **1999**, *50*, 781−785.
(16) Talapatra, S. K.; Amarendra, P.; Bani, T. *J. Indian Chem. Soc*. **1978**, *55*, 1152−1160.
(17) Boulware, R. T.; Stermitz, F. R. *J. Nat. Prod*. **1981**, *44*, 200−205.
(18) Tandon, S.; Rastogi, P. *Phytochemistry* **1976**, *15*, 1789−1791.
(19) Hanuman, J. B.; Mishra, A. K.; Sabata, B. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1181−1185.
(20) Achenbach, H.; Waibel, R.; Addaemensah, I. *Phytochemistry* **1983**, *22*, 749−753.
(21) Ghogomu-Tih, R.; Bodo, B.; Nyasse, B.; Sondengam, B. L. *Planta Med*. **1985**, 464.
(22) Alfatafta Ali, A.; Musa, H.; Zarga, A.; Salim, S.; Sabri, F. A. J.; Shamma, M. *J. Nat. Prod*. **1989**, *52*, 818−821.
(23) Pettit, G. R.; Singh, S. B. *Can. J. Chem*. **1987**, *65*, 2390−2396.
(24) Lino von Poser, G.; Schripsena, J.; Henriques, A. T.; Rosendal, J. S. *Biochem. Syst. Ecol*. **2000**, *28*, 351−366.
(25) Sticher, O. *New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutical Activity*; Wagner, H., Wolff, P., Eds.; Springer: New York, 1977; pp 145−176.
(26) Ismailogu, U. B.; Saracoglu, I.; Harput, U. S.; Sahin-Erdemli, I. *Ethnopharmacology* **2002**, *79*, 193−197.
(27) Chai-Ming, L.; Yang, J.-J.; Wang, P.-Y.; Lin, C.-C. *Planta Med*. **2000**, *66*, 374−377.
(28) Prasad, K. *Drug News Perspect*. **2000**, *13*, 99−104.
(29) Lee, S. K.; Mbwambo, Z. H.; Chung, H. S.; Luyengi, L.; Gamez, E. J. C.; Mehta, R. J.; Kinghorn, A. D.; Pezzuto, J. M. *Comb. Chem. High Throughput Screening* **1998**, *1*, 35−46.
(30) Tiwari, A. K. *Curr. Sci*. **2001**, *81*, 1179−1187.
(31) Tiwari, A. K. *Curr. Sci.* **2004**, *86*, 1092−1102.
(32) Rao, R. J.; Tiwari, A. K.; Kumar, U. S.; Reddy, S. V.; ALI, Z. A.; Rao, J. M. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 2777−2780.

NP058036Y