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Antimicrobial constituents from the rhizomes of *Rheum emodi*

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Abstract

The bioassay-guided chemical examination of the rhizomes of *R. emodi* resulted in the isolation of two new oxanthrone esters, revandchinone-1, revandchinone-2, a new anthraquinone ether revandchinone-3 and a new oxanthrone ether, revandchinone-4. Their structures were established based on spectroscopic and degradative evidence. Occurrence of oxanthrone ether is reported for the first time. The anti bacterial and anti fungal activity of the isolates is studied.

Keywords: *Rheum emodi*; Rhubarb; Polygonaceae; Revandchinone-1; Revandchinone-2; Revandchinone-3; Revandchinone-4; Chrysophanol; Physcion; β-Asarone

1. Introduction

Rheum emodi is an important medicinal plant, which finds an extensive use in Ayurvedic and Unani systems of medicine (Wealth of India, 1972). As part of our programme on new bioactive molecules from the plants used in traditional systems of medicine, we have taken up the phytochemical investigation of the rhizomes of *R. emodi* procured from the Himalayan region of India. A recent publication (Matsuda et al., 2001) deals with a variety of biological properties of the compounds isolated from various Rheum species. These biological properties viewed together with the fact that *Rheum emodi* is a plant that is hitherto not so well studied prompted a reinvestigation of this plant. The petroleum ether and chloroform extracts of the rhizomes showed a moderate degree of antibacterial and antifungal activities. The bioactive petroleum ether extract of the rhizomes thus yielded two novel oxanthrone esters revandchinone-1 (**1**), revandchinone-2 (**2**) and β-asarone. The chloroform extract yielded a new anthraquinone ether, revandchinone-3 (**3**) and a novel oxanthrone ether, revandchinone-4 (**4**). The presence of β-asarone in *Rheum* genus is reported for the first time. The names revandchinone-1, revandchinone-2, revandchinone-3 and revandchinone-4 are proposed for the new compounds. In addition to these new compounds, chrysophanol, physcion and emodin were isolated.

2. Results and discussion

The petrol extract of the rhizomes of *R. emodi* on repeated column chromatography yielded two new compounds **1** and **2** in addition to β-asarone, chrysophanol and physcion.

Compound (**1**), revandchinone-1, was isolated as bright yellow needles, mp 214 °C. The molecular formula $C_{34}H_{46}O_6$, was deduced from the positive ion FAB MS $(M+H)^+$ 551. The UV spectrum displayed bands at 440 and 285 nm suggesting an anthrone system. The IR spectrum showed absorption bands at 3441 cm^{-1} (–OH), 1625 cm^{-1} (chelated-C=O) and 1749 cm^{-1} (ester–C=O). The 200 MHz ^1H NMR spectrum ($CDCl_3$) indicated the presence of two singlets at δ 12.10 and 12.30, which are characteristic of two chelated hydroxyl groups. Two doublets at δ 7.35 and δ 6.65 ($J=3$ Hz) each integrating for one proton of H-4 and H-2 and two singlets at δ 7.65 and δ 7.10 for H-5 and H-7 were char-

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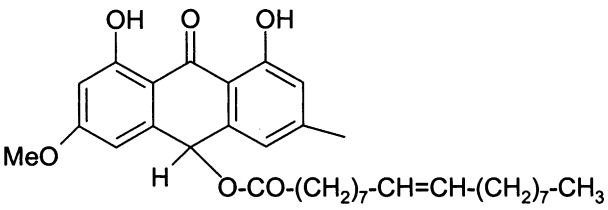
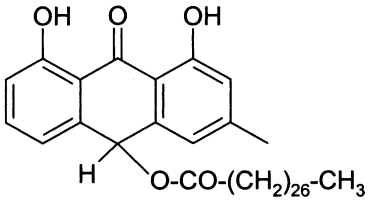


Revandchinone-1 (**1**)



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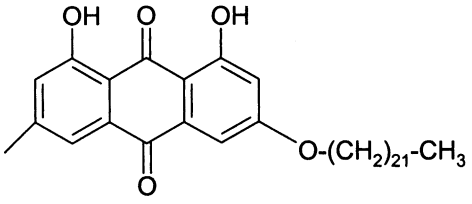

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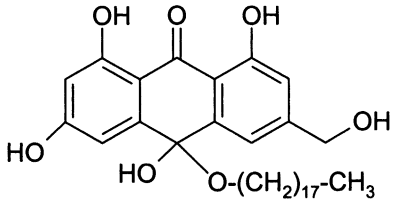

Revandchinone-4 (**4**)

acteristic of an anthraquinone system. The singlet at δ 2.45 integrating for three protons was characteristic of an aromatic methyl group of an anthraquinone. The presence of an aromatic methoxyl group was indicated by a three proton singlet at δ 3.98. The one proton multiplet at δ 4.10 for the oxymethine proton at C-10 of an oxanthrone in addition to a triplet at δ 2.35 for a –OCOC$\underline{H}_2$– group is characteristic of an oxanthrone ester (Anu and Rao, 2001). A broad singlet at δ 1.15–1.40 integrating for 22 protons is characteristic of methylene groups belonging to an aliphatic chain, the triplet at δ 5.30 (2H) for the olefinic protons and the two multiplets at δ 2.00 (2H) and δ 1.60 (2H) for the two methylenes adjacent to olefinic carbons demonstrates that this oxanthrone is an unsaturated aliphatic ester. Thus **1** was tentatively identified as a derivative of physcion-9-anthrone with an unsaturated aliphatic side chain at C-10 position.

The EI-MS of compound (**1**) gave the molecular weight of the corresponding anthraquinone, physcion, with M^+ 284 due to cleavage of the side chain to form the oxonium ion and hence the anthraquinone (Monache et al., 1987). The structure was further confirmed by alkaline hydrolysis of compound (**1**), which yielded oleic acid. Oleic acid was methylated and compared by Co-TLC and GLC with an authentic sample of methyloleate. Thus (**1**) was identified as 1,8-dihydroxy-3-methyl-6-methoxy-9(10H)-anthracenone-10-oxyoleate.

Compound (**2**), revandchinone-2 was isolated as orange yellow needles, mp 201 °C. The molecular formula $C_{43}H_{66}O_5$ was deduced from the positive ion FAB MS $(M+H)^+$ 663. The UV spectrum displayed bands at 440 and 290 nm. The IR spectrum indicated bands at 3445 cm^{-1} (–OH), 1629 cm^{-1} (chelated–C=O) and 1744 cm^{-1} (ester–C=O). The 200 MHz ^1H NMR spectrum is similar to that of (**1**) except for the protons of the aliphatic side chain. The mass spectra (EIMS and FABMS) coupled with the integration of the protons in the aliphatic region indicated the compound (**2**) to be 1,8-dihydroxy-3-methyl-9(10H)-anthracenone-10-oxy-octacosanoate. The structure of revandchinone-2 (**2**) was further confirmed by alkaline hydrolysis of **2** which yielded an acid, identified as octacosanoic acid by ^1H NMR and EIMS.

The bioactive chloroform extract on column chromatography yielded two new compounds **3** and **4** in addition to chrysophanol, physcion and emodin.

Compound (**3**), was isolated as yellow needles, mp 220 °C. The molecular formula $C_{37}H_{54}O_5$ was deduced from the positive ion FABMS $(M+Na)^+$ 601. The UV spectrum displayed bands at 440 and 285 nm suggesting an anthrone system. The IR spectrum showed absorption bands at 3445 cm^{-1} (–OH), 1676 (–C=O), 1627 cm^{-1} (chelated C=O) and 1275 cm^{-1} (suugestive of an ether linkage). The 200 MHz ^1H NMR spectrum (MeOH-d_4 + $CDCl_3$) indicated the presence of two meta coupled protons at δ 7.18 (J = 2.5Hz) and 6.54 (J = 2.5 Hz) and another set of meta coupled protons at δ 7.52 (J = 2.0 Hz) and 6.98 (J = 2.0 Hz) resembling the emodin skeleton (Wang et al., 1996). The singlet at δ 2.39 integrating for three protons was characteristic of an aromatic methyl group. A triplet at δ 0.88 integrating for three protons and a broad singlet at δ1.18–1.30 integrating for 40 protons coupled with a triplet at δ 4.15 (–O–CH_2–) indicated an aliphatic side chain with an ether linkage.

The ^{13}C NMR spectrum of compound (**3**) indicated the presence of 12 aromatic carbons in addition to an aromatic methyl group and a terminal methyl group of an aliphatic chain. The chelated carbonyl carbon at δ 189.9 and another carbonyl carbon at δ 181.2 are typical of an anthraquinone moiety. The DEPT spectrum of

compound **3** indicated the presence of two methyl carbons and four methine carbons.

All the above data clearly suggest that compound (**3**) is a derivative of emodin where in a long aliphatic chain is attached to one of the hydroxyl groups. The ^{1}H NMR spectrum in DMSO-d_6 clearly shows the presence of two chelated hydroxyl groups at δ 12.05 and 12.15 clearly suggesting the aliphatic chain is attached to the hydroxyl group present at C-6 in emodin. Thus compound (**3**) was identified as 3-decosyloxy-1,8-di hydroxyl-6-methyl-9,10-dihydro-9,10-anthracenedione.

Compound (**4**), was isolated as yellow needles, mp 235 °C. The molecular formula $C_{33}H_{48}O_7$ was deduced from the positive ion FABMS $(M+Na)^+$ of 579. The UV spectrum indicated bands at 440 and 285 nm suggesting an anthrone system. The IR spectrum indicated absorption bands at 3445 cm^{-1} (–OH), 1626 cm^{-1} (chelated C=O) and 1277 cm^{-1} (suggestive of an ether linkage). The 300 MHz ^{1}H NMR spectrum (DMSO-d_6) indicated the presence of two chelated hydroxyl groups at δ 12.00 and 12.15. In addition, it also showed the presence of four aromatic protons at δ 7.25, 7.35, 7.60 and 7.85 assigned to H-2, H-7, H-4 and H-5 for the anthraquinone moiety. The singlet at δ 4.65 integrating for two protons indicated the presence of a –CH_2OH group. A triplet at δ 0.88 for a terminal methyl group and a broad singlet at δ 1.20–1.45 integrating for 32 protons for the methylene protons of an aliphatic side chain coupled with a broad un-resolved triplet at δ 4.20 showed the presence of an ether linked aliphatic chain.

The ^{13}C NMR spectrum of compound (**4**) showed the presence of 12 aromatic carbons and one chelated carbonyl group. The notable feature of the ^{13}C NMR spectrum was the down field shift of C-4 and C-5 carbons and appearance of C-10 carbon at δ 120.19 which clearly indicated that C-10 carbon was attached to two oxygen atoms. This fact was further confirmed by DEPT experiment which suggested that C-10 carbon was a quarternary carbon. Further, the DEPT spectrum showed the presence of four methine signals and one methyl carbon. Based on these observations, the structure of compound (**4**) was confirmed as 1,3,8,10-tetrahydroxy-6-hydroxymethyl-10-octadecyloxy-9,10-di hydro-9-anthracenone.

Revandchinone-1 and Revandchinone-2 belong to a relatively new class of compounds known as oxanthrone esters. Recently four new oxanthrone esters namely, kleinioxanthrone-1 and 2 (Anu and Rao, 2001) and kleinioxanthrone-3 and 4 (Anu and Rao, 2002) have been isolated from *Cassia kleinii* in our laboratory. The biogenetic precursors for these compounds could be the corresponding oxanthrones. The oxanthrone could then have been esterified easily in the plant, leading to the formation of oxanthrone esters. The rare occurrence of the only oxanthrone (Hemlata, 1994) may thus be explained.

Revandchinone-4 is the first report of the occurrence of an oxanthrone ether which could have been formed by the attack of oxyanion of the aliphatic alcohols on the unchelated carbonyl carbon of the corresponding anthraquinone.

According to two recent communications, chrysophanol, physcion and emodin are known to possess antibacterial (Hatano et al., 1999) and antifungal (Agarwal et al., 2000) properties. Hence, the bioassay of the new compounds was carried out and the results are summarized in Tables 1 and 2. Revandchinone-1 and 3 showed only a moderate degree of antibacterial activity. On the other hand, revandchinone-4 was found to be a good antibacterial substance with an inhibition zone dia. of 14 mm at 100 μg/ml test conc. The three compounds, revandchinone-1, 3 and 4 all exhibited a moderate degree of antifungal activity. The bioassay of revandchinone-2 (**2**) could not be carried out as the compound was isolated in extremely low quantities.

3. Experimental

3.1. General experimental procedures

^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded using TMS as internal standard. Mass spectra were recorded on a VG Auto Spec-M (FAB MS, EI MS). IR spectra were recorded on a Nicolet spectro-

Table 1
Antibacterial activity levels of compounds **1**, **3** and **4**

Microorganism (Gram +ve)	Compd **1**		Compd **3**		Compd **4**		Control pen G (30 μg)
	(30 μg)	(100 μg)	(30 μg)	(100 μg)	(30 μg)	(100 μg)	
Bacillus subtilis	7	8	7	8	7	7	20
Bacillus sphaericus	8	9	7	8	7	7	19
Staphylococcus aureus	7	8	7	9	12	14	18
Gram −ve							Streptomycin (30 μg)
Klebsiella aerogenes	7	7	7	7	7	7	30
Chromobacterium violaceum	7	8	7	8	7	8	28
Pseudomonas aeruginosa	–	–	–	–	7	7	34

Inhibitory zone diameters are in mm.

Table 2
Antifungal activity levels of compounds **1**, **3** and **4**

Microorganism	Compd **1**		Compd **3**		Compd **4**		Control (clotrimazole)
	100 μg	150 μg	100 μg	150 μg	100 μg	150 μg	100 μg
Rhizopus oryzae	8	9	8	9	8	9	23
Aspergillus niger	10	11	10	11	9	11	22

Inhibitory zone diameters are in mm.

meter. UV spectra were recorded on a GBC Cintra 10e spectrometer.

3.2. Plant material

Rheum emodi rhizomes procured commercially from the Himalayan region, were authenticated by Professor K.V.B.R. Tilak, former Head, Division of Microbiology, Indian Agricultural Research Institute, New Delhi. A voucher specimen of the sample was deposited at organic chemistry division-1, IICT, Hyderabad, India.

3.3. Bacteria

Six test organisms, *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenes* (MTCC 39), *B. sphaericus* (MTCC 511) and *Chromobacterium violaceum* (MTCC 2656) were obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on nutrient agar slants and were subcultured in Petri dishes prior to testing.

3.4. Fungi

Two test organisms *Rhizopus oryzae* (MTCC 262) and *Aspergillus niger* (MTCC 281) were obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on potato dextrose agar slants and were subcultured in Petri dishes prior to testing.

The nutrient agar and the potato dextrose agar media were procured from M/S Himedia, Mumbai, India.

3.5. Extraction and isolation

Dried and powdered rhizomes (500 g) of the plant were extracted (Soxhlet) successively with petroleum ether (60–80 °C) and chloroform. The petrol extract was filtered to remove chrysophanol. The filtrate was evaporated and the residue was twice chromatographed on a silica gel (100–200 mesh) column. All the compounds **1**, **2**, and β-asarone were obtained with hexane:EtOAc (99:1) as eluents. In addition to these compounds chrysophanol, physcion and emodin were also obtained. Compound **1** and **2** were further purified by preparative TLC.

The chloroform extract was concentrated and the residue was chromatographed over a silica gel column (100–200 mesh). Compound **3** was obtained with hexane: EtOAc (90:10) as eluent and compound **4** was obtained with hexane: EtOAc (85:15) as eluent.

Chrysophanol: Orange needles (EtOAc): mp 192–193 °C. The physiochemical data are identical with reported literature (Banville et al., 1974, Ros Kelly and Chandra Kumar, 1983). Physcion: Orange needles (hexane); mp 207 °C. The physiochemical data are identical with reported literature (Wang et al., 1996, Tiwari and Anjali, 1979).

Revandchinone-1 (**1**) (0.022 g) was obtained as yellow needles (EtOAc): mp 214 °C; UV (MeOH) λ_{max} 440, 285 nm. IR (KBr) γ_{max} 3441 (OH), 1625 (chelated-CO), 1749 (ester-CO) cm^{-1}. ^{13}C NMR (50 MHz $CDCl_3$) δ 162.5 (C-1), 106.7 (C-2), 165.2 (C-3), 108.2 (C-4), 124.5 (C-5), 148.4 (C-6), 121.2 (C-7), 166.5 (C-8), 190.0 (C-9), 69.2 (C-10), 133.20 (C-11), 113.2 (C-12), 110.2 (C-13), 135.3 (C-14), 56.1 (-OMe), 14.5 (C-18′), 22.1 (-CH_3), 181.5 (C-1′), 31.9 (C-2′), 22.6-29.6 (C-3′-8′, C-11′-17′), 34.5 (C-9′), 33.0 (C-10′). FAB MS [M + 1] 551. EI MS 284 (25), 232 (20), 149 (35), 85 (47), 57 (100).

Revandchine-2 (**2**) (0.010 g) was obtained as orange yellow needles (EtOAc), mp 201 °C; UV (MeOH) λ_{max} 440, 290 nm. IR (KBr) γ_{max} 3445 (OH), 1629 (chelated-CO), 1744 (ester-CO) cm^{-1}. ^{13}C NMR (50 MHz $CDCl_3$) δ 162.4 (C-1), 115.7 (C-2), 149.1 (C-3), 124.4 (C-4), 124.1 (C-5), 136.8 (C-6), 119.7 (C-7), 162.2 (C-8), 192.4 (C-9), 68.3 (C-10), 133.0 (C-11), 123.1 (C-12), 121.1 (C-13), 133.5 (C-14), 22.4 (-CH_3), 181.7 (C-1′), 31.6 (C-2′), 21.7–29.5 (C-3′-27′), 13.8 (C-28′). FAB MS [M + 1] 663. EI MS 254 (10), 204 (10), 104 (100).

3.6. Hydrolysis of oxanthrone esters

The two compounds **1** and **2** (8 mg) were taken in 2.5% alcoholic KOH (5 ml) refluxed for 1 h until an aliquot showed the disappearance of the starting material on TLC. The resulting solution was then cooled and water (5 ml) was added. The hydrosylate was neutralised with dil. HCl and extracted with ether, dried (Na_2SO_4) and evaporated to obtain oleic acid and octacosanoic acids respectively.

3.7. Methylation of oleic and octacosanoic acids

The acids were taken in MeOH (5 ml) and thionylchloride was added and refluxed for 3 h. Then MeOH was removed and the residue was dissolved in EtOAc washed with bicarbonate and water, dried (Na_2SO_4) and evaporated to obtain the methyl ester. The methyl ester of oleic acid was identified by Co-TLC and GLC with an authentic sample and by ^1H NMR and EI MS for ester obtained on methylation of octacosanoic acid.

Revandchinone −3 (**3**) (0.05 g) was obtained as yellow needles (EtOAc), mp 220 °C. UV (MeOH) λ_{max} 440, 280 nm. IR (KBr) γ_{max} 3445 (OH), 1627 (chelated-CO), 1676 cm^{-1}, 1275 (ether linkage) cm^{-1}., ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.5 (C-1), 107.3 (C-2), 165.5 (C-3), 108.5 (C-4), 124.0 (C-5), 148.0 (C-6), 120.3 (C-7), 164.2 (C-8), 189.9 (C-9), 181.2 (C-10), 132.8 (C-11), 113.1 (C-12), 109.0 (C-13), 135.0 (C-14), 22.2 (Ar-CH_3), 34.2 (C-1′), 31.2 (C-2′), 22.6–29.3 (C-3′-C-21′), 14.3 (C-22′). FABMS $(M + Na)^+$ 601.

Revandchinone −4 (4) (0.038 g) was obtained as yellow needles (EtOAc), mp 235 °C. UV (MeOH) λ_{max} 440, 285 nm. IR (KBr) γ_{max} 3445 (OH), 1626 (chelated-CO), 1277 (ether linkage) cm^{-1}, ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.5 (C-1), 109.4 (C-2), 162.8 (C-3), 125.2 (C-4), 134.2 (C-5), 154.6 (C-6), 121.5 (C-7), 163.0 (C-8), 182.3 (C-9), 120.1 (C-10), 134.0 (C-11), 126.5 (C-12), 117.9 (C-13), 138.0 (C-14), 62.9 (-CH_2OH), 49.4 (C-1′), 32.0 (C-2′), 31.3 (C-3′), 22.0–29.8 (C-4′-C-17′), 14.7 (C-18′). FABMS $(M+Na)^+$ 579.

β-Asarone (5). UV (MeOH) λ_{max} 305 nm. IR (KBr) γ_{max} 1608, 1580, 1508 (aromatic) and 857 (*cis*-double bond) cm^{-1}. ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6 (C-3′), 56.2, 56.5, 56.8 (-OMe), 96.1 (C-3), 114.7 (C-6), 118.5 (C-5), 125.0 (C-1′), 125.6 (C-2′), 142.7 (C-1), 148.8 (C-2), 151.7 (C-4). EI MS M^+ 208 (100), 193 (50), 165 (45), 140 (10), 69 (15).

All the data are in agreement with the literature values (Patra and Mitra, 1981).

3.8. Anti bacterial assay of compounds 1, 3 and 4

The ready made nutrient agar medium (23 g) was suspended in distilled water (1000 ml) and heated to boiling until it dissolved completely. The medium and the Petri dishes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. Stock solutions were prepared by dissolving plant extract in DMSO and different concentrations were made (30 μg and 100 μg).

Agar cup bioassay was employed for testing antibacterial activity of plant extract following the standard procedure (Linday, 1962). The medium was poured in to Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 ml of 24 h old culture of test organism was inoculated. After inoculation, cups were scooped out with 6mm sterile cork borer and the lids of the dishes were replaced. To each cup different concentrations of test solutions (30, 100 μg) were added. Controls were maintained with DMSO and penicillin G (for Gram + ve bacteria) and Streptomycin (for Gram-ve bacteria). The treated and the controls were kept in an incubator at 37 °C for 24 h to 48 h. Inhibition zones were measured and diameter was calculated. Three to four replicates were maintained for each treatment.

3.9. Anti fungal assay of compounds 1, 3 and 4

The method followed for anti fungal bioassay is similar to that followed for anti bacterial assay where in the medium is potato dextrose agar 39 g/l and the control is clotrimazole. Also, the treated and the controls were kept at RT for 24–96 h and inhibition zones were measured and diameter was calculated.

References

Agarwal, S.K., Singh, S.S., Verma, S., Kumar, S., 2000. Anti fungal activity of anthraquinone derivatives from *Rheum emodi*. J. Ethno. Pharmacol. 72 (1), 43.

Anu, S.J., Rao, J.M., 2002. Oxanthrone esters from the roots of *Cassia kleinii*. Phytochemistry 59, 425.

Anu, S.J., Rao, J.M., 2001. Oxanthrone esters from the aerial parts of *Cassia kleinii*. Phytochemistry 57, 583.

Banville, J., Grandmaison, J., Lang, G., Brassard, P., 1974. Reactions of keten acetals. Part I. A simple synthesis of some naturally occurring anthraquinones. Can. J. Chem. 52, 80.

Hatano, T., Uebayashi, H., Ito, H., Shiota, S., Tsuchiya, T., Yoshida, T., 1999. Phenolic constituents of *Cassia* seeds and anti bacterial effects of some naphthalenes and anthraquinones on methicillin-resistant *Staphylococcus aureus*. Chem. Pharm. Bull. 47 (8), 1121.

Hemlata, K.S.D., 1994. Proceedings of Indian National Science Academy, Part A 60 (6), 765.

Linday, M. E., 1962. Practical introduction to microbiolog., E & F. N. Spon Ltd. p 177.

Mastuda, H., Morikawa, T., Toguchida, I., Park, J., Harima, S., Yoshikawa, M., 2001. Antioxidant constituents from Rhubarb. Structural requirements of stilbenes for the activity and structures of two new anthraquinone glucosides. Bioorg. Med. Chem. 9, 41.

Monache, G.D., Monache, F.D., Benedetto, R.D., Ogukwa, J.U., 1987. New metabolites from *Psorospermum tenuifolium*. Phytochemistry 26 (9), 2611.

Patra, A., Mitra, A.K., 1981. Constituents of *Acorus calamus*: structure of acoramone. ^{13}C NMR spectra of *cis*- and *trans*- asarone. J. Nat. Prod. 44, 668.

Ross Kelly, T., Chandra Kumar, N.S., 1983. Practical procedure for the isolation of emodin and chrysophanol. J. Org. Chem. 48, 3573.

Tiwari, R.D., Anjali, R., 1979. Chemical examination of the seeds of *Cassia laevigata*. J. Ind. Chem. Soc. 56 (9), 942.

Wang, E.C., Shih, M.H., Liu, M.C., Chen, M.T., Lee, G., 1996. Studies on constituents of *Saururus chinensis*. Heterocycles 43, 969.

Wealth of India, Raw Materials, Vol. 9. CSIR New Delhi, 1972.