Isolation and Structures of Novel Fungal Depsidones, Emeguisins A, B, and C, from *Emericella unguis* [1]

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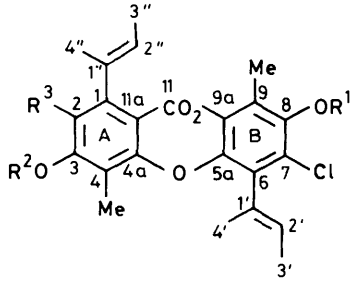
Three novel depsidones designated emeguisins A (**1**), $C_{23}H_{23}ClO_5$, B (**2**), $C_{24}H_{25}ClO_5$, and C (**3**), $C_{24}H_{24}Cl_2O_5$, were isolated from the mycelium of *Emericella unguis*, strain IFM 42017. Their structures were determined on the basis of spectroscopic and chemical investigations of these compounds and their derivatives. This is the first example of depsidones bearing two 1-methylprop-1-enyl groups in one molecule having been isolated. It is also interesting to note that 3-ethyl-5,7-dihydroxy-3,6-dimethylphthalide (**7**) was isolated from the same fungus, in view of the probable biogenesis of the above three depsidones.

Recently we reported the isolation of fungal depsidones, 2-chlorounguinol (**4**), unguinol (**5**), and nidulin (**6**), along with phthalide (**7**) from the culture filtrate of *Emericella unguis* Malloch & Cain, an ascosporic strain IFM 42017.[2] In the course of our search for related compounds, three new fungal depsidones, emeguisins A (**1**), B (**2**), and C (**3**), were isolated from the chloroform extract of the mydelium of the aforementioned fungus. The structural elucidation of emeguisins A (**1**), B (**2**), and C (**3**) is described in this paper.

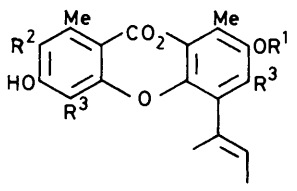
Results and Discussion

Emeguisin A (**1**) and emeguisin B (**2**) both gave a positive colouration (green) on Beilstein's test and gave molecular ions at *m/z* 414 and 416, and 428 and 430, respectively, in the ratio 3:1. These results suggest the presence of one chlorine atom in both compounds (**1**) and (**2**). The elemental analyses of (**1**) and (**2**) confirmed the empirical formulae as $C_{23}H_{23}ClO_5$ and $C_{24}H_{25}ClO_5$, respectively. Emeguisin B (**2**) showed a ^{1}H n.m.r. signal at δ_H 3.85 assigned to the aromatic methoxy protons and a signal at δ_H 5.67 assigned to the phenolic hydroxy proton, whereas emeguisin A (**1**) showed no methoxy signals but two phenolic hydroxy protons at δ_H 5.29 and 5.68 in its ^{1}H n.m.r. spectrum. Other ^{1}H n.m.r. signals of compounds (**1**) and (**2**) were similar. Compound (**1**) gave compound (**2**) by methylation with diazomethane. These results confirm that the emeguisins A (**1**) and B (**2**) have the same carbon skeletons and that (**2**) is a monomethylated derivative of (**1**).

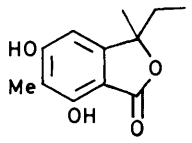
Kamal *et al.* obtained corresponding orsellinic acid derivatives from the depsidones (**8**) and (**9**) by the process of the basic hydrolysis followed by ether cleavage with a mixture of hydriodic acid and nitric acid.[3] Thus emeguisin B (**2**) was



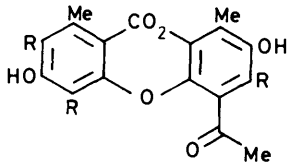
(**1**) $R^1 = R^2 = R^3 = H$
(**2**) $R^1 = R^3 = H, R^2 = Me$
(**3**) $R^1 = Me, R^2 = H, R^3 = Cl$
(**11**) $R^1 = R^2 = Me, R^3 = H$



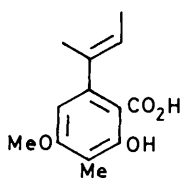
(**4**) $R^1 = R^3 = H, R^2 = Cl$
(**5**) $R^1 = R^2 = R^3 = H$
(**6**) $R^1 = Me, R^2 = R^3 = Cl$



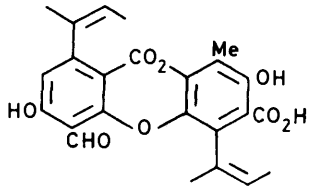
(**7**)



(**8**) R = H
(**9**) R = Cl



(**10**)



(**12**)

Table 1. ^{13}C N.m.r. chemical shifts of emeguisins and related compounds in $CDCl_3$

Carbon	(1)	(2)	(3)[a]	(6)	(11)
1	135.86	136.62	134.62	140.24	136.65
2	112.54	107.65	117.61	119.69	107.74
3	158.02	161.07	158.77[b]	157.32	160.95
4	113.62	115.79	116.09	110.34	115.98
4a	161.97	161.16	155.42[b]	151.95	161.15
5a	143.39	143.44	145.58	145.44	146.35
6	130.10	130.11	129.58	129.48	130.17
7	115.13	115.01	122.94	124.44	123.69
8	149.60	149.65	151.97	152.70	152.00
9	116.03	115.95	123.07	123.53	123.34
9a	142.91	143.01	142.03	141.78	142.66
11	164.37	163.93	161.63	161.59	163.70
11a	112.64	112.87	113.95	115.97	112.75
1-Me				18.88	
4-Me	8.53	8.82	9.62[c]		8.87
9-Me	9.93	9.96	9.88[c]	10.46	10.35
3-OMe		55.94			55.93
8-OMe			60.25	60.53	60.53
1′	134.24	134.32	135.14	136.09	135.73
2′	127.99	127.96	127.75	128.23	127.76
3′	13.98[b]	13.97[b]	13.53[d]	14.18	13.98[b]
4′	17.44[c]	17.46[c]	17.06[e]	17.52	17.47[c]
1″	147.41	147.34	145.26		149.74
2″	125.21	125.04	124.62		125.03
3″	14.33[b]	14.38[b]	13.59[d]		14.38[b]
4″	17.68[c]	17.93[c]	17.18[e]		17.97[c]

[a] This compound was measured in $(CD_3)_2SO$ at 80 °C. [b–e] The assignments may be reversed.

refluxed with 2M sodium hydroxide, and we obtained compound (**10**), $C_{13}H_{16}O_4$. The i.r. absorption maxima at 3 000—2 600 and 1 630 cm^{-1} and ^{13}C n.m.r. signal at δ_C 174.06 showed the presence of a chelated carboxylic acid in structure (**10**). The ^1H n.m.r. signal at δ_H 11.78, exchangeable with D_2O, for compound (**10**) was assigned to a phenol attached to the carbon (C-2) next to the carbon bearing the carboxylic acid. From the above results, it was confirmed that compound (**10**) was a derivative of salicylic acid, and was derived from the A ring moiety of compound (**2**).

The seven protons at δ_H 1.81 (3 H), 1.97 (3 H), and 5.63 (1 H) in compound (**10**) were shown to couple with the four carbons at δ_C 14.03, 18.88, 123.83, and 148.20. The coupling patterns of these signals suggested the presence of a 1-methylprop-1-enyl group in structure (**10**). The carbon signal at δ_C 148.20, assigned to C-1 of the 1-methylprop-1-enyl group, was extremely downfield in comparison with the corresponding signal in nidulin (**6**) (δ_C 136.09, Table 1). So it is proposed that the 1-methylprop-1-enyl group in compound (**10**) is attached to the carbon (C-6) next to the carbon bearing the carboxylic acid. The other functional groups of compound (**10**) are one methyl and one methoxy group as judged from the ^1H and ^{13}C n.m.r. spectra. Since the carbon signal at δ_C 162.23, assigned to the carbon bearing the hydroxy group, appeared as a sharp quartet, the aromatic methyl group should be attached to the position vicinal to the hydroxy group. The signal for the carbon bearing the carboxy group (δ_C 102.75) was observed as a doublet (*J* 7 Hz), so the aromatic proton was undoubtedly located *meta* to the carboxylic acid. When the methoxy protons at δ_H 3.87 in compound (**10**) were irradiated, a 12% nuclear Overhauser enhancement of the proton signal of δ_H 6.20 was observed. All of the above results confirmed the structure of compound (**10**) as 2-hydroxy-4-methoxy-3-methyl-6-(1-methylprop-1-enyl)-benzoic acid, which was the A ring moiety of emeguisin B (**2**).

The functional groups of the B ring of emeguisin B (**2**) were a methyl, a hydroxy, a 1-methylprop-1-enyl group, and a chlorine atom, from the elemental analysis and ^1H and ^{13}C n.m.r. spectra. The A and B rings of structure (**2**) were connected *via* ester and ether functions, on consideration of the presence of the carbon signal assigned to an ester (δ_C 163.93) and the co-occurrence of compounds (**4**), (**5**), and (**6**) from this fungus. The assignments of the carbon chemical shifts (Table 1) and the determination of the position of the functional groups in the B ring of compound (**2**) were carried out mainly on the basis of the signal multiplicity in the proton-coupled ^{13}C n.m.r. spectrum and the additivity rule of the substituent effects on the benzene ring.[4] Thus the structure of emeguisin B was assigned as (**2**).

Emeguisin B (**2**) was methylated with iodomethane and sodium carbonate to give emeguisin B methyl ether (**11**), $C_{25}H_{27}ClO_5$, which had no hydroxy group on consideration of its i.r. and ^1H n.m.r. spectra. Fujita *et al.* reported[5] the *O*-methylation effect on the ^{13}C n.m.r. chemical shifts of *ortho*-disubstituted phenols. The *O*-methylation shift values (Δ) in Table 2 were calculated by subtracting the carbon chemical shifts of phenols [(**2**) in this case] from the corresponding chemical shifts of their methyl ethers [(**11**) in this case]. The above shift values of the B ring, which are listed in Table 2 (example 1), were consistent with those of simple *ortho*-disubstituted phenols.* In particular, *O*-methylation [from (**2**) to (**11**)] caused downfield shifts for *ipso*-, *ortho*-, and *para*-carbon atoms. The other aromatic carbons of compounds (**2**) and (**11**), including those in the A ring, shifted by less than 0.4 p.p.m. The above results confirmed the structure of emeguisin B as (**2**), and the structure of emeguisin A was also confirmed as (**1**) since this is the demethylation product of compound (**2**).

Emeguisin C (**3**) gave a positive colouration (green) on Beilstein's test and gave molecular ions at *m/z* 462, 464, and 466 in the proportions ~9:7:1. These results suggested the presence of two chlorine atoms in compound (**3**). The elemental analysis of compound (**3**) confirmed the molecular formula as $C_{24}H_{24}Cl_2O_5$. Emeguisin C (**3**) showed the signals of two 1-methylprop-1-enyl groups, two aromatic methyls, one methoxy, and one phenol in the ^1H and ^{13}C n.m.r. spectra. The absorption maximum at 1 720 cm^{-1} in the i.r. spectrum and the ^{13}C n.m.r. signal at δ_C 161.63 suggested the presence of an ester function in structure (**3**). The above data suggested that emeguisin C (**3**) was the chlorinated compound of emeguisin B (**2**) at its aromatic hydrogen.

In order to determine the structure of emeguisin C (**3**), chlorination of emeguisin B (**2**) was attempted, but no products other than the starting material and/or degraded products, which were hard to purify, were obtained. Therefore, the determination of the positions of the functional groups of compound (**3**) was carried out mainly on the basis of the signal multiplicity in the proton-coupled ^{13}C n.m.r. spectrum and the additivity rule of the substituent effects on the benzene ring.[4] Unexpectedly, it was confirmed that the methoxy group (δ_C 60.25) in compound (**3**) was located at C-8 in the B ring, the same as for nidulin (**6**), but different from emeguisin B (**2**).

In order to determine the position of the functional groups in emeguisin C (**3**), we at first applied the *O*-methylation effects of *ortho*-disubstituted phenols[5] to the B ring between emeguisin B (**2**) and nidulin (**6**). The *O*-methylation shift values (Δ) on the carbons in the B ring were calculated and are listed in Table 2 (example 2). These values were consistent with those of *ortho*-disubstituted phenols.* The shifts of the carbons in the A ring between compounds (**2**) and (**6**) were less than 0.5 p.p.m. Thus, the *O*-methylation effect of *ortho*-disubstituted phenols can also

* Average shift values of *O*-methylation in ref. 5: +2.5 p.p.m. for *ipso*-carbon, +5.3 p.p.m. for *ortho*-carbon, and +4.5 p.p.m. for *para*-carbon. The *meta*-carbons are not greatly affected by *O*-methylation.

Table 2. ^{13}C N.m.r. chemical shifts of B ring carbons of some depsidones

Example	Compound and shift value (Δ)	C-5a	C-6	C-7	C-8	C-9	C-9a
1	(**2**)	143.44	130.11	115.01	149.65	115.95	143.01
	(**11**)	146.35	130.17	123.69	152.00	123.34	142.66
	Δ	+2.91	+0.06	+8.68	+2.35	+7.39	−0.35
2	(**2**)	143.44	130.11	115.01	149.65	115.95	143.01
	(**6**)	145.44	129.48	124.44	152.70	123.53	141.78
	Δ	+2.00	−0.63	+9.43	+3.05	+7.58	−1.23
3	(**1**)	143.39	130.10	115.13	149.60	116.03	142.91
	(**3**)	145.58	129.58	122.94	151.97	123.07	142.03
	Δ	+2.19	−0.52	+7.81	+2.37	+7.04	−0.88

be applied to the B ring carbons of the depsidones differing, in the nature of the substituents, from the A ring carbons. Then, we calculated the shift values between emeguisin A (**1**) as a phenol and emeguisin C (**3**) as a methyl ether as listed in Table 2 (example 3). The characteristic downfield shifts of carbon atoms *ipso*, *ortho*, and *para* to the methylated hydroxy group were comparable with those of the *ortho*-disubstituted phenols.* From these results, it was confirmed that the methoxy group of emeguisin C (**3**) was located at C-8, and thus the structure of the B ring of emeguisin C (**3**) was determined.

The carbon signal at δ_C 145.26, assigned to C-1 of the 1-methylprop-1-enyl group in the B ring of emeguisin C (**3**), was extremely downfield in comparison with the corresponding signal in the A ring side-chain at δ_C 135.14, so the 1-methylprop-1-enyl group of the B ring was attached to the carbon (C-1) next to the carbon bearing the carbonyl group of the lactone. Two ^{13}C n.m.r. signals at δ_C 155.42 and 158.77, which were assigned to the carbons bearing an oxygen function, both appeared as quartets in the proton-coupled spectrum. Therefore, the carbon bearing the methyl group in the B ring was attached next to the above two carbons. The above results confirmed the location of the functional groups in the B ring and consequently the structure of emeguisin C was determined as (**3**).

The double bonds in the 1-methylprop-1-enyl groups of emeguisins A (**1**), B (**2**), and C (**3**) were assumed to be of *Z*-configuration between two methyl groups, in consideration of the co-occurrence of 2-chlorounguinol (**4**), unguinol (**5**), and nidulin (**6**), but this problem has not been completely determined as yet. The *X*-ray structural analysis will be attempted to determine these configurations.

It is very interesting that depsidones with two 1-methylprop-1-enyl groups in the molecule, emeguisins A (**1**), B (**2**), and C (**3**), were isolated from *E. unguis* accompanied by the depsidones which possess one 1-methylprop-1-enyl residue, 2-chlorounguinol (**4**), unguinol (**5**), and nidulin (**6**), on consideration of the probable biogenesis. The A ring and the B ring of emeguisins would be derived basically from the same compound, 2,4-dihydroxy-3-methyl-6-(1-methylprop-1-enyl)benzoic acid. The only compound, (**12**), from *Sirodesmium diversum* was reported by P. J. Curtis[6] as having two 1-methylprop-1-enyl residues, but no data were given. The biosynthesis of emeguisins will be studied in due course.

Emeguisins A (**1**) and B (**2**) had monoamine oxidase inhibitory ratios of only 4.7% and 3.6%, respectively, at a concentration of 10^{-6} mol dm^{-3}, by Kraml's method.[7] The antibacterial activities of emeguisins A (**1**), B (**2**), and C (**3**) were tested against *Bacillus subtilis* (Ehrenberg) Cohn and *Escherichia coli* (Migula) Castellani & Chalmers.[8] Emeguisin A (**1**) inhibited the growth of *B. subtilis*, a Gram-positive bacterium, at a concentration of 50 μg $disc^{-1}$.

* As on p. 2612.

Experimental

M.p.s were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Electron impact (e.i.) mass spectra were taken with a JEOL JMS-D 300 spectrometer. U.v. spectra and i.r. spectra were recorded on a Hitachi 124 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^1H N.m.r. (99.60 MHz) spectra were recorded on a JEOL JNM-FX 100 spectrometer, while ^{13}C n.m.r. (100.43 MHz) spectra were taken with a JEOL JNM-GX 400 spectrometer, with tetramethylsilane as internal standard. The coupling patterns are indicated as follows: singlet = S or s, doublet = D or d, triplet = T or t, quartet = Q or q, multiplet = m, and broad = br. Capital letters refer to the pattern resulting from directly bonded coupling ($^1J_{C,H}$). Column chromatography was performed on Kieselgel 60 (Art. 7734; Merck). Low-pressure liquid chromatography (l.p.l.c.) was performed on a Chemco Low-Prep pump 81-M-2 and a glass column (200 × 10 or 20 mm) packed with silica gel CQ-3 (30—50 μm, Wako). T.l.c. was conducted on pre-coated Kieselgel 60 F_{254} (Art. 5715; Merck). Spots on t.l.c. were detected by their absorption under u.v. light and/or with iodine vapour.

Isolation of Metabolites from the mycelium of Emericella unguis.—*Emericella unguis*, strain IFM 42017, was cultivated at 28 °C for 3 weeks in Czapek-Dox medium containing 0.2% yeast extract, using 60 Roux flasks containing 250 ml of the above medium in each flask. The dried mycelia (78 g) were extracted with chloroform at room temperature, and the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue (9.0 g) was chromatographed on silica gel with chloroform to give emeguisin B (**2**) (300 mg) and nidulin (**6**) (2.0 g), with chloroform–methanol (100:1) to give the fraction mentioned below, and with chloroform–methanol (20:1) followed by l.p.l.c. with chloroform as solvent to give unguinol (**5**) (80 mg). The fraction eluted with chloroform–methanol (100:1) was rechromatographed on silica gel with benzene–acetone (100:1) followed by l.p.l.c. with benzene as solvent to give emeguisin A (**1**) (200 mg), and with benzene–acetone (50:1) to give ergosterol (50 mg), 2-chlorounguinol (**4**) (200 mg), and 3-ethyl-5,7-dihydroxy-3,6-dimethylphthalide (**7**) (20 mg). The mother liquor, after filtration from compound (**2**), was purified (l.p.l.c., cyclohexane) to obtain emeguisin C (**3**) (130 mg).

2-Chlorounguinol (**4**), unguinol (**5**), nidulin (**6**), and 3-ethyl-5,7-dihydroxy-3,6-dimethylphthalide (**7**) were identified with samples obtained from the culture filtrate of this fungus.[2]

Emeguisin A (**1**) was obtained as needles (from benzene), m.p. 185—187 °C (Found: C, 66.8; H, 5.6. $C_{23}H_{23}ClO_5$ requires C, 66.59; H, 5.59%); *m/z* 414 and 416 (M^+, 38 and 13%), 398 and 400 [$(M - 16)^+$, 16 and 6], and 378 [$(M - HCl)^+$, 16]; $\lambda_{max.}$(EtOH) 280 nm (log ε 4.14); $\nu_{max.}$(KBr) 3 400 (OH), 1 720, and 1 700 cm^{-1} (CO_2); $\delta_H(CDCl_3)$ 1.72 [3 H, br d, *J* 6.8 Hz,

C(Me)=CH*Me*], 1.83 [3 H, br d, *J* 6.8 Hz, C(Me)=CH*Me*], 1.89 [3 H, br s, C(*Me*)=CHMe], 1.94 [3 H, br s, C(*Me*)=CHMe], 2.18 (3 H, s, ArMe), 2.30 (3 H, s, ArMe), 5.29 (1 H, s, phenolic OH), 5.49 [2 H, br q, *J* 6.8 Hz, C(Me)=C*H*Me], 5.68 (1 H, s, phenolic OH), and 6.50 (1 H, s, ArH). ^{13}C N.m.r. signals are summarized in Table 1.

Emeguisin B (**2**) was obtained as needles (from methanol), m.p. 204—206 °C (Found: C, 67.15; H, 5.8. $C_{24}H_{25}ClO_5$ requires C, 67.21; H, 5.88%); *m/z* 428 and 430 (M^+, 100 and 37%), 413 and 415 [$(M - 15)^+$, 38 and 14], and 393 [$(M - Cl)^+$, 39]; $\lambda_{max.}$(EtOH) 276 nm (log ε 4.09); $\nu_{max.}$(KBr) 3 400 (OH) and 1 720 cm^{-1} (CO_2); δ_H($CDCl_3$) 1.75 [3 H, br d, *J* 6.8 Hz, C(Me)=CH*Me*], 1.83 (3 H, br d, *J* 6.8 Hz, C(Me)=CH*Me*], 1.94 [3 H, br s, C(*Me*)=CHMe], 1.95 [3 H, br s, C(*Me*)=CHMe], 2.14 (3 H, s, ArMe), 2.30 (3 H, s, ArMe), 3.85 (3 H, s, OMe), 5.50 [2 H, br q, *J* 6.8 Hz, C(Me)=C*H*Me], 5.67 (1 H, s, phenolic OH), and 6.53 (1 H, s, ArH). ^{13}C N.m.r. signals are summarized in Table 1.

Emeguisin C (**3**) was obtained as a viscous oil, (Found: C, 62.4; H, 5.2. $C_{24}H_{24}Cl_2O_5$ requires C, 62.22; H, 5.22%); *m/z* 462, 464, and 466 (M^+, 100, 70, and 14%), 447, 449, and 451 [$(M - 15)^+$, 40, 27, and 7], 427 and 429 [$(M - Cl)^+$, 78 and 30], and 399 and 401 [$(M - Cl - 28)^+$, 77 and 28]; $\lambda_{max.}$(EtOH) 220sh (log ε 4.55) and 264sh nm (3.87); $\nu_{max.}$(NaCl) 3 400 (OH) and 1 720 cm^{-1} (CO_2); δ_H($CDCl_3$) 1.72 [3 H, dq, *J* 6.9 and 1.0 Hz, C(Me)=CH*Me*], 1.84 [3 H, br d, *J* 6.4 Hz, C(Me)=CH*Me*], 1.96 [3 H, br, C(*Me*)=CHMe], 2.03 [3 H, br s, C(*Me*)=CHMe], 2.25 (3 H, br s, ArMe), 2.29 (3 H, s, ArMe), 3.79 (3 H, s, OMe), 5.22 [1 H, br q, *J* 6.9 Hz, C(Me)=C*H*Me], 5.50 [1 H, br, C(Me)=C*H*Me], and 6.14 (1 H, s, phenolic OH); δ_H[$(CD_3)_2SO$; 80 °C] 1.65 [3 H, dq, *J* 6.9 and 1.0 Hz, C(Me)=CH*Me*], 1.80 [3 H, dq, *J* 6.7 and 1.0 Hz, C(Me)=CH*Me*], 1.93 [3 H, br s, C(*Me*)=CHMe], 1.99 [3 H, br s, C(*Me*)=CHMe], 2.20 (3 H, s, ArMe), 2.21 (3 H, s, ArMe), 3.75 (3 H, s, OMe), 5.11 [1 H, qq, *J* 6.9 and 1.0 Hz, C(Me)=C*H*Me], 5.43 [1 H, qq, *J* 6.7 and 1.0 Hz, CH(Me)=C*H*Me], and 6.51 (1 H, s, phenolic OH). ^{13}C N.m.r. signals are summarized in Table 1.

Methylation of Emeguisin A (**1**) *with Diazomethane*.—A solution of diazomethane in ether was added to emeguisin A (**1**) (5 mg) and the mixture was left at room temperature for 1 day. The reaction mixture was evaporated and the residue was chromatographed on silica gel with chloroform to give crystals (3 mg). This compound was identified with emeguisin B (**2**) by comparison of t.l.c. behaviour, ^{1}H n.m.r. and i.r. spectra, and mixed m.p.

Methylation of Emeguisin B (**2**) *with Iodomethane and Sodium Carbonate*.—Sodium carbonate (200 mg, pulverized) and iodomethane (1.5 ml) were added to a solution of emeguisin B (**2**) (50 mg) in acetone (1.0 ml) and the mixture was refluxed for 1.5 h. The reaction mixture was poured into ice–water, acidified with dil. hydrochloric acid to pH 2, extracted with ethyl acetate, and the extract dried (Na_2SO_4). Evaporation gave a residue, which was purified by l.p.l.c. (cyclohexane as solvent) to afford an *emeguisin B monomethyl ether* (*emeguisin A dimethyl ether*) (**11**) (40 mg) as a viscous oil (Found: C, 67.9; H, 6.15. $C_{25}H_{27}ClO_5$ requires C, 67.79; H, 6.14%); *m/z* 442 and 444 (M^+, 100 and 38%), 427 and 429 [$(M - 15)^+$, 35 and 13], and 407 [$(M - Cl)^+$, 62]; $\lambda_{max.}$(MeOH) 271 nm (log ε 4.05); $\nu_{max.}$(KBr) 1 740 cm^{-1} (CO_2); δ_H($CDCl_3$) 1.75 [3 H, dd, *J* 6.8 and 1.0 Hz, C(Me)=CH*Me*], 1.83 [3 H, dd, *J* 6.8 and 1.0 Hz, C(Me)=CH*Me*], 1.94 [6 H, br s, C(*Me*)=CHMe × 2], 2.15 (3 H, s, ArMe), 2.32 (3 H, s, ArMe), 3.79 (3 H, s, OMe), 3.85 (3 H, s, OMe), 5.47 [1 H, qq, *J* 6.8 and 1.0 Hz, C(Me)=C*H*Me], 5.53 [1 H, qq, *J* 6.8 and 1.0 Hz, C(Me)=C*H*Me], and 6.55 (1 H, s, ArH). ^{13}C N.m.r. signals are listed in Table 1.

Alkaline Hydrolysis of Emeguisin B (**2**).—Emeguisin B (**2**) (150 mg) was suspended in 2M sodium hydroxide (5 ml) and the mixture was refluxed for 2 h, then poured into ice–water, and extracted with ethyl acetate at pH 2. The extract was washed with water and dried (Na_2SO_4). The residue obtained on evaporation was purified by l.p.l.c. (benzene as solvent) to obtain *4-methoxy-3-methyl-6-(1-methylprop-1-enyl)salicylic acid* (**10**) (30 mg) as needles (from cyclohexane), m.p. 144—146 °C (Found: C, 66.25; H, 6.85. $C_{13}H_{16}O_4$ requires C, 66.08; H, 6.83%); *m/z* 236 (M^+, 58%), 218 [$(M - H_2O)^+$, 60], and 203 [$(M - H_2O - Me)^+$, 100]; $\lambda_{max.}$(MeOH) 222 (log ε 4.39), 265 (4.04), and 303 nm (3.63); $\nu_{max.}$(KBr) 3 450 (OH), 3 000—2 600 (CO_2H), and 1 630 (chelated CO); δ_H($CDCl_3$) 1.81 [3 H, dq, *J* 6.6 and 1.0 Hz, C(Me)=CH*Me*], 1.97 [3 H, br s, C(*Me*)=CHMe], 2.09 (3 H, s, ArMe), 3.87 (3 H, s, OMe), 5.63 [1 H, br q, *J* 6.6 Hz, C(Me)=C*H*Me], 6.20 (1 H, s, ArH), and 11.78 (1 H, s, phenolic OH); δ_C($CDCl_3$) 7.94 (Q, *J* 127 Hz, 3-Me), 14.03 [Qd, *J* 124 and 4 Hz, C(Me)=CH*Me*], 18.88 [Qd, *J* 126 and 7 Hz, C(*Me*)=CHMe], 55.73 (Q, *J* 143 Hz, OMe), 102.75 (d, *J* 7 Hz, C-1), 104.69 (D, *J* 158 Hz, C-5), 112.20 (m, C-3), 123.83 [Dm, *J* 148 Hz, C(Me)=*C*HMe], 138.95 (m, C-6), 148.20 [m, *C*(Me)=CHMe], 162.23 (q, *J* 4 Hz, C-2), 162.32 (m, C-4), and 174.06 (S, CO_2H).

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