

FOUR NEW PHENOLIC CONSTITUENTS FROM LICORICE (ROOT OF *GLYCYRRHIZA* SP.)

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Abstract. — Four new isoprenoid-substituted phenolic constituents, semilicoisoflavone B (1), isoangustone A (2), 1-methoxyficifolinol (3), and licoriphenone (4), along with nine known phenolic compounds, were isolated from a commercial licorice (root of *Glycyrrhiza* sp.) and their structures were elucidated on the basis of spectroscopic and chemical means.

Licorice, the root of various species of *Glycyrrhiza* (Leguminosae), is one of the most important Chinese traditional medicines and used frequently also in Japan. Besides its triterpenoidal constituent, glycyrrhizin, many isoprenoid-substituted phenolic constituents have been hitherto reported.¹⁻³ In connection with the studies on the crude drugs effective on visceral larva migrans,⁴ we are interested in the chloroform extract of licorice. Here we add four new phenolic constituents, semilicoisoflavone B (1), isoangustone A (2), 1-methoxyficifolinol (3), and licoriphenone (4), which were isolated from the chloroform extract of a commercial licorice,⁵ after several chromatographical separations (normal and reversed phased), along with nine known phenolic compounds, (+)-licoricidin,⁶ (+)-7-*O*-methyllicoricidin,^{7,8} (-)-medicarpin,⁹ (-)-1-*O*-methylphaseollidin,¹⁰ isoglycyrol,^{3,11} 3-*O*-methylglycyrol,^{3,12} kumatakenin,¹³ licoflavonol,¹³ and gancaonin I.³

Compound 1 (semilicoisoflavone B), C₂₀H₁₆O₆, was obtained as colorless needles, mp 131-134°C. The uv absorption maximum at 265 nm (log ϵ = 4.43) and the ir absorption at 1655 cm⁻¹ suggested that it is an isoflavone derivative.¹⁴ Its ¹H nmr spectrum showed two sets of *meta*-coupled aromatic protons (δ 6.21 and 6.37, and 6.72 and 6.90, each d, *J* = 2.0 Hz), a proton on a γ -pyrone ring (δ 8.31, s), and signals of

2,2-dimethylpyran ring [δ 1.40 (6H, s), 5.74 and 6.37 (each 1H, d, $J=9.5$ Hz)], whose pattern was identical with that of gancanin H (5) except that 1 lacked the signals of one prenyl moiety.³ The ^{13}C nmr of 1 was in good agreement with that of licoisoflavone B¹⁵ (6) for rings A and C, and with that of 5 for rings B and D (Table I), thus proving the structure 1. Since this compound is isomeric to licoisoflavone B on the position of a hydroxyl group on ring B, the name, semilicoisoflavone B, is proposed.

Compound 2 (isoangustone A), $\text{C}_{25}\text{H}_{26}\text{O}_6$, colorless needles, mp 191-193°C, was also an isoflavone derivative (uv: 272 nm, ir: 1642 cm^{-1}). In the ^1H nmr spectrum, it showed the signals of following protons: 1) two prenyl groups at δ 1.63 (3H, br s), 1.68 (6H, br s), 1.73 (3H, br s), 3.23 and 3.24 (each 2H, d, $J=7.3$ Hz), 5.18 and 5.29 (each 1H, t with fine splittings, $J=7.3$ Hz), 2) three aromatic protons at δ 6.43 (s), 6.65 and 6.87 (each d, $J=2.1$ Hz), 3) a proton on a γ -pyrone ring at δ 8.20 (s), and 4) a hydrogen-bonded (δ 13.00) and non-bonded hydroxyl groups (δ 8.29, 9.32). The pattern of the aromatic protons was very similar to that of gancanin H (5).³ The ^{13}C nmr spectrum of 2 was in good agreement with that of 5 for rings A and C and a prenyl group on ring A (Table I). The chemical shifts of ring B carbons of 2 were also similar to those of 5 and 1, indicating that they have the same substitution pattern for ring B, i.e. oxygen functions at C-3' and C-4', and an prenyl group at C-5'. Thus the structure 2 was assigned to this compound and the name isoangustone A is proposed, because it is isomeric to angustone A (7).¹⁵

Compound 3 (1-methoxyficifolinol), $\text{C}_{26}\text{H}_{30}\text{O}_5$, colorless needles, mp 128-132°C, was optically active, $[\alpha]_D -173^\circ$ (CHCl_3). Its ^1H nmr spectrum showed signals of protons characteristic of pterocarpan,^{16,17} [δ ca. 3.25 (overlapped with other signals), 3.51 (1H, t, $J=10.7$ Hz), 4.14 (1H, dd, $J=10.7, 4.9$ Hz), 5.53 (1H, d, $J=6.7$ Hz), along with two prenyl groups [δ 1.64, 1.695, 1.698, 1.75 (each 3H, br s), 3.20-3.35 (4H, m), 5.25 and 5.31 (each 1H, t with fine splittings)], a methoxyl (δ 3.89, s), three singlet aromatic protons (δ 6.23, 6.33, 7.02), and two hydroxyl groups (δ 8.19 and 8.55). The ^{13}C nmr spectrum of 3 supported the above assignments. The position of substituents on the pterocarpan ring was determined as follows. Hydrogenation of 3 over 30% Pd-C in EtOH-AcOH gave, with concomitant hydrogenolysis of ring D, an isoflavan 8, whose mass spectrum exhibited intense retro-Diels-Alder peaks¹⁸ at m/z 223 and 206, which are corresponding to

Table I. ^{13}C Nmr Data of New Phenolic Compounds from Licorice and Related Compounds

Carbon*	2 ^a	5 ^{a, d}	1 ^a	6 ^{a, e}	3 ^{b, h} (*)	4 ^c (*)	10 ^{a, f} (*)	12 ^g (*)
Rings A and C								
2	153.4	153.7	154.0	155.8	66.9(6)	-----		
3	121.1	122.1	122.1	120.7	39.9(6a)	33.3(8)		
4	180.2	180.1	180.0	180.8	76.2(11a)	204.7(7)		202.6(7)
4a	104.3	104.3	104.5	104.8	107.0(11b)	113.0(1)		113.9(1)
5	158.8	158.9	162.0	162.0	160.8(1)	164.1(2)		165.1(2)
6	110.9	111.2	98.9	99.1	115.9(2)	103.6(3)		103.5(3)
7	161.8	162.0	164.2	164.3	158.1(3)	166.3(4)		165.1(4)
8	92.8	93.0	93.6	93.8	99.9(4)	108.6(5)		108.6(5)
8a	155.3	155.3	157.5	157.9	155.7(5)	134.0(6)		133.9(6)
Ring B							(Ring A)	
1'	122.6	122.9	122.6	111.4	118.7(6b)	106.6(1')	106.9(6)	
2'	120.4	122.0	121.9	151.3	125.6(7)	158.6(2')	157.0(5)	
3'	144.4	145.1	145.1	110.0	120.4(8)	115.9(3')	113.3(4a)	
4'	143.1	140.1	140.0	153.8	156.1(9)	157.0(4')	153.0(8a)	
5'	127.9	121.3	121.3	107.6	98.1(10)	97.4(5')	98.9(8)	
6'	114.0	117.6	117.4	128.7	159.4(10a)	155.3(6')	154.5(7)	
Prenyl and 2,2-dimethylpyran								
9	21.0	21.0			23.2(1')	23.0(1'')	22.4(9)	
10	122.2	122.2			124.4(2')	123.7(2'')	123.7(10)	
11	130.5	130.5			130.6(3')	130.9(3'')	129.1(11)	
12	17.6	17.6			17.8(4')	17.6(4'')	17.6(12)	
13	25.4	25.3			25.9(5')	25.7(5'')	25.4(13)	
7'	28.2	117.1	116.9	117.1	28.6(1'')			
8'	123.0	131.2	131.1	131.5	124.7(2'')			
9'	130.8	75.9	75.9	75.5	131.3(3'')			
10'	17.6	27.5	27.4	27.6	17.9(4'')			
11'	25.5	27.5	27.4	27.6	25.9(5'')			
Methoxyl					62.9	55.6(4'-OMe) 62.2(2'-OMe)		

a) In DMSO- d_6 .b) In acetone- d_6 .c) In chloroform- d .

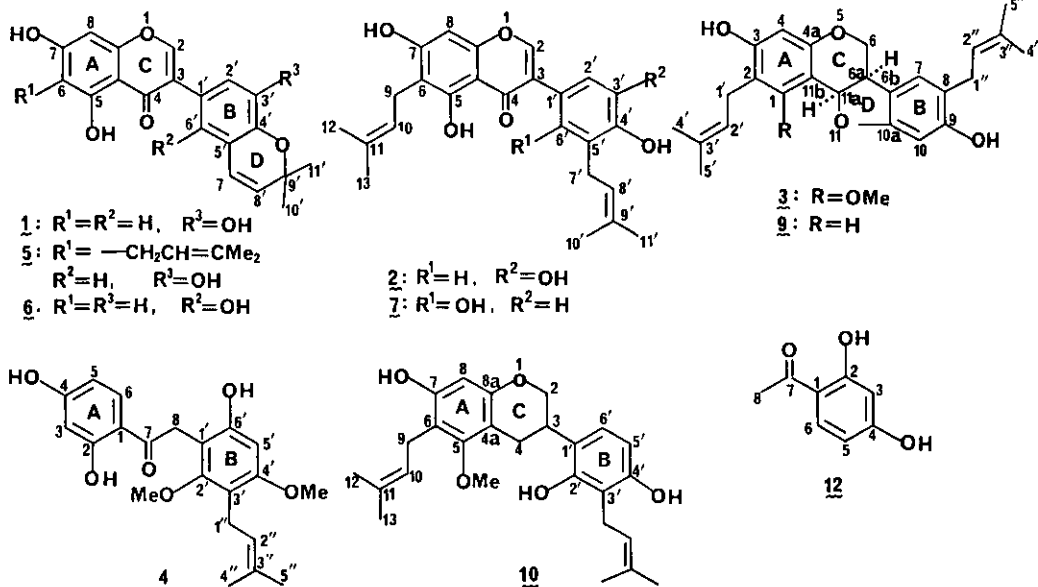
d) Data from ref. 3.

e) Data from ref. 15.

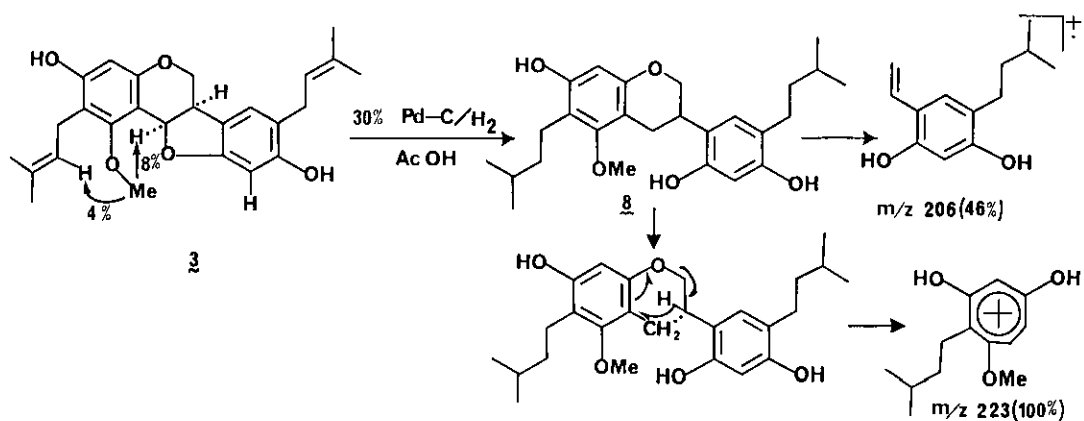
f) Data from ref. 6.

g) Data (in dioxane/ CS_2) from "Atlas of ^{13}C -NMR Data," Vol. 1, No. 676.h) Assignments were confirmed by 1J and $^2,^3J$ C-H Cosy experiments.

* Isoflavone numbering is adopted. Real numberings of the compounds are indicated in parentheses.

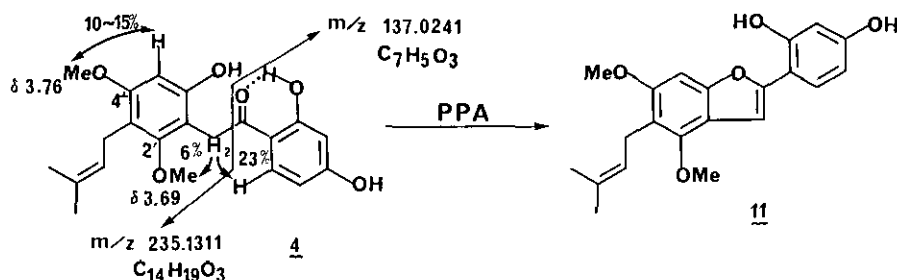


rings A and ring B of 8, respectively. This indicated that ring A of 3 bears a methoxyl, a prenyl, and a hydroxyl groups and ring B bears a prenyl and a hydroxyl groups. Since all the aromatic protons appeared as singlet, a prenyl and a hydroxyl groups on ring B have to be placed at C-8 and C-9. Chemical shifts of aromatic protons of 3 in CDCl₃ were very similar to those of ficifolinol¹⁹ (9) except that 3 lacked H-1 signal and that H-4 signal shifted to upfield (δ 6.25) compared to that of 9 (δ 6.32). This suggests that 3 has the same substitution pattern as that of 9 except that 3 has an additional oxygen function at C-1, which must be a methoxy group. This was confirmed by nuclear Overhauser effect (nOe) experiments. Irradiation of the methoxyl proton (δ 3.89) resulted in nOe's on H-11a at δ 5.53 (8%) and the olefinic proton of a prenyl group at δ 5.25 (4%). Thus compound 3 was determined as 1-methoxyficifolinol.²⁰



Compound 4 (licoriphenone), C₂₁H₂₄O₆, colorless needles, mp 136-138°C, has a chelated carbonyl group (ir: 1630 cm⁻¹, ¹³C nmr: δ 204.7). The ¹H nmr spectrum of 4 showed a singlet methylene signal at δ 4.18, along with a 1,2,4-trisubstituted benzene [δ 6.34 (1H, d, J=2.4 Hz), 6.43 (1H, dd, J=8.9, 2.4 Hz), and 8.09 (1H, d, J=8.9 Hz)], a pentasubstituted benzene [δ 6.36 (1H, s)], a prenyl group [δ 1.65, 1.74 (each 3H), 3.26 (2H, br d, J=6.5 Hz), 5.15 (1H, t with fine splittings, J=6.5 Hz)], two methoxyl groups (δ 3.69 and 3.76), and a chelated hydroxyl group (δ 12.28, br s). In combination with these data, the uv spectrum of 4 (278 nm, log ϵ =4.18 and 315 nm, log ϵ =3.88) was ascribed to the phenylacetophenone structure. The ¹³C nmr of 4 indicated the presence of resacetophenone (12) structure in 4 and the chemical shifts of the remaining carbons were similar to those of ring A carbons of licoricidin⁶ (10) (Table 1), suggesting that ring B of 4 has phloro-

glucinol-type oxygen substituents. The intense ms fragment peaks at m/z 235.1311 ($C_{14}H_{19}O_3$) and 137.0241 ($C_7H_5O_3$) confirmed that ring A bears two hydroxyl groups and ring B bears two methoxyl, one prenyl, and one hydroxyl groups. The substituent positions on ring B were established as follows. Chemical shifts of the methoxyl carbons (δ 55.6 and 62.2) indicated that one of the OMe groups (δ 62.2) has diortho-substituents,²¹ suggesting that this OMe group to be at C-2'. Irradiation of the OMe signal at δ 3.76 produced 15% nOe on the proton at δ 6.36 (H-5'), while irradiation of the latter proton resulted in 10% nOe on the former protons. On the other hand, irradiation of the methylene protons at δ 4.18 (H-8) produced nOe's on the OMe at δ 3.69 (6%) and the aromatic proton (H-6) at δ 8.09 (23%). Thus the structure 4 was assigned for this compound, which must be the precursor of gancanin I (II).²² In fact, treatment of 4 with polyphosphoric acid in CH_2Cl_2 produced II. To our present knowledge, such phenylacetophenone derivative is the first occurrence in licorice, and the name licoriphenone is proposed.



Of the thirteen phenolic compounds above isolated, licoflavonol showed appreciable nematocidal activity against the larvae of *Toxocara canis*, while the others were weakly or not at all active. These will be reported elsewhere.

EXPERIMENTAL

Unless otherwise stated, uv spectra are taken in EtOH and given by λ_{max} nm ($\log \epsilon$), ir spectra in KBr disks and ν_{max} are given by cm^{-1} , and EI-ms are taken at 20 eV and major peaks are given by m/z .

Isolation of New Compounds from Commercial Licorice

Cut licorice (1 kg) was extracted with boiling $CHCl_3$ and the extract was chromatographed on silica gel eluting with $CHCl_3$ and $CHCl_3$ -MeOH (1:1). The latter fraction was divided into three fractions by Sephadex LH-20 column eluting with MeOH. The middle fraction (12 g) was re-chromatographed on silica gel to yield frs. A,

B, and C. Repeated chromatographies of fr. A on silica gel and polyamide column gave 1-methoxyficifolinol (3) (120 mg) together with licoricidin, medicarpin, and 7-O-methyllicoricidin. Chromatographies of fr. B on silica gel and a recycling preparative hplc (LC-908) yielded isoangustone A (2) (33 mg), licoriphenone (4) (28 mg), and semilicoisoflavone B (1) (70 mg) together with the other known compounds indicated in the text.

Semilicoisoflavone B (1)

Compound 1 crystallizes from chloroform as colorless needles, mp 131-134°C. Uv: 265 (4.43), 330 (3.66). Ir: 1655, 1620. EI-MS (probe): 352[M]⁺ (81), 339 (17), 338 (21), 337 (100), 311 (10), 299 (13), 187 (11), 82 (16). High-resolution-MS (HR-MS), *m/z*: 352.0960 [M]⁺ (C₂₀H₁₆O₆ requires: 352.0946). ¹H Nmr (DMSO-*d*₆): δ 1.40 (6H, br s, H-10' and H-11'), 5.74 (1H, d, J=9.5 Hz, H-8'), 6.21, 6.37 (each 1H, d, J=2 Hz, H-6 and H-8), 6.37 (1H, d, J=9.5 Hz, H-7'), 6.72, 6.90 (each 1H, d, J=2.0 Hz, H-2' and H-6'), 8.31 (1H, s, H-2).

Isoangustone A (2)

Compound 2 crystallizes from ether-hexane as colorless needles, mp 191-193°C. Uv: 272 (4.44). Ir: 1642, 1630. EI-MS: 422[M]⁺ (98), 405 (21), 380 (28), 379 (81), 368 (31), 367 (100), 366 (22), 323 (41), 311 (42), 221 (25), 204 (21), 165 (30). HR-MS, *m/z*: 422.1720 [M]⁺ (C₂₅H₂₆O₆ requires: 422.1727). ¹H Nmr (DMSO-*d*₆): δ 1.63 (3H, br s, H-13), 1.68 (6H, br s, H-12 and H-10'), 1.73 (3H, br s, H-11'), 3.23, 3.24 (each 2H, d, J=7.3 Hz, H-9 and H-7'), 5.18 (1H, t with fine splittings, J=7.3 Hz, H-10), 5.29 (1H, t with fine splittings, J=7.3 Hz, H-8'), 6.43 (1H, s, H-8), 6.65 (1H, d, J=2.1 Hz, H-2'), 6.87 (1H, d, J=2.1 Hz, H-6'), 8.20 (1H, s, H-2), 8.29, 9.32, 13.00 (each 1H, br s, OH x 3).

1-Methoxyficifolinol (3)

Compound 3 crystallizes from chloroform as colorless needles, mp 128-132°C. [α]_D -173° (c=3, CHCl₃). Uv: 234 (sh 4.30), 287 (3.98). Ir: 1625, 1598, 1490. EI-MS: 422[M]⁺ (100), 421 (12), 368 (7), 367 (26), 366 (9), 351 (9), 311 (7), 221 (5), 191 (7), 161 (6). HR-MS, *m/z*: 422.2087 [M]⁺ (C₂₆H₂₆O₅ requires 422.2091). ¹H Nmr (acetone-*d*₆): δ 1.64, 1.695, 1.698, 1.75 (each 3H, br s, H-4', H-5', H-4'', H-5''), 3.20-3.35 (5H, m, H-6, H-1', H-1''), 3.51 (1H, t, J=10.7 Hz, H-6), 3.89 (3H, s, OMe), 4.14 (1H, dd, J=10.7, 4.9 Hz, H-6a), 5.25 (1H, t with fine splittings, J=7.0 Hz, H-2'), 5.31 (1H, t with fine splittings, J=7.0 Hz, H-2''), 5.53 (1H, d, J=6.7 Hz, H-11a), 6.23 (1H, s, H-4), 6.33 (1H, s, H-10), 7.02 (1H, s, H-7), 8.19 and 8.55

(each 1H, br s, OH x 2). ^1H Nmr (CDCl_3): δ 1.76, 1.770, 1.773, 1.83 (each 3H, s, H-4', H-5', H-4'', H-5''), 3.28, 3.39 (each 2H, br d, $J=6.5$ Hz, H-1' and H-1''), 3.34 (1H, m, H-6), 3.59 (1H, t, $J=10.9$ Hz, H-6), 3.92 (3H, s, OMe), 4.16 (1H, dd, $J=10.9, 5.2$ Hz, H-6a), 5.24, 5.29 (each 1H, t with fine splittings, $J=6.5$ Hz, H-2' and H-2''), 5.30 (1H, br s, OH), 5.57 (1H, br s, OH), 5.59 (1H, d, $J=6.4$ Hz, H-11a), 6.25 (1H, s, H-4), 6.33 (1H, s, H-10), 6.94 (1H, s, H-7).

Hydrogenation of 1-Methoxylicifolinol (3)

Compound 3 (5 mg) was hydrogenated with H_2 (4 kg/cm 2) over 30% Pd-C in ethanol-acetic acid (2:1, 15 ml) for 6 h to give isoflavan 8 as an oil (3 mg). EI-MS: 428 [$\text{M}]^+$ (38), 371 (26), 315 (10), 224 (15), 223 (100), 207 (10), 206 (46), 205 (24), 193 (19), 167 (29).

Licoriphenone (4)

Compound 4 crystallizes from ether-hexane as colorless needles, mp 136-138°C. ^1H Nmr (CDCl_3): δ 1.65 (3H, d, $J=0.9$ Hz), 1.74 (3H, br s), 3.26 (2H, br d, $J=6.5$ Hz, H-1''), 3.69 (3H, s, 2'-OMe), 3.76 (3H, s, 4'-OMe), 4.18 (2H, s, H-8), 5.15 (1H, t with fine splittings, $J=6.5$ Hz, H-2''), 6.02 (1H, br s, OH), 6.34 (1H, d, $J=2.4$ Hz, H-3), 6.36 (1H, s, H-5'), 6.43 (1H, dd, $J=2.4, 8.9$ Hz, H-5), 7.76 (1H, br s, OH), 8.09 (1H, d, $J=8.9$ Hz, H-6), 12.28 (1H, br s, OH).

Conversion of Licoriphenone (4) to Gancaonin I (11)

A mixture of 4 (3 mg) and polyphosphoric acid (12 mg) in dry methylene chloride (2 ml) was heated at 50°C for 20 min. Methanol (10 ml) was added to the reaction mixture and the whole was concentrated to dryness. The residue was purified by preparative tlc (silica gel, benzene-acetone=5:1) to give 11 (1.5 mg), which was identical with gancaonin I (tlc and ^1H nmr comparisons).

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REFERENCES AND NOTES

1. S. Shibata and T. Saitoh, *J. Indian Chem. Soc.*, 1978, **55**, 1184 and references

cited therein.

2. T. Hatano, T. Yasuhara, K. Miyamoto, and T. Okuda, *Chem. Pharm. Bull.*, 1988, **36**, 2286.
3. T. Fukai, Q. Wang, T. Kitagawa, K. Kusano, T. Nomura, and Y. Iitaka, *Heterocycles*, 1989, **29**, 1761.
4. F. Kiuchi, X. Chen, and Y. Tsuda, *Shoyakugaku Zasshi*, 1989, **43**, 42.
5. The licorice was purchased from Hokuriku Yakugyo Co. Ltd., Kanazawa, Japan.
The original plant may be ascribed to *G. uralensis*, though it was not rigidly identified.
6. T. Fukai, M. Toyono, and T. Nomura, *Heterocycles*, 1988, **27**, 2309.
7. US pat. 4,639,466 (*Chem. Abstr.*, 1987, **106**, 189006c).
8. T. L. Smith, M. J. Wyvratt, and H. Mrozik, *J. Org. Chem.*, 1987, **52**, 2029.
9. Y. Sakagami, S. Kumai, and A. Suzuki, *Agr. Biol. Chem.*, 1974, **38**, 1031.
10. N. W. Preston, *Phytochemistry*, 1977, **16**, 2044.
11. T. Shiozawa, S. Urata, T. Kinoshita, and T. Saitoh, *Chem. Pharm. Bull.*, 1989, **37**, 2239.
12. T. Saitoh and S. Shibata, *Chem. Pharm. Bull.*, 1969, **17**, 729.
13. T. Saitoh, T. Kinoshita, and S. Shibata, *Chem. Pharm. Bull.*, 1976, **24**, 1242.
14. T. Kinoshita, T. Saitoh, and S. Shibata, *Chem. Pharm. Bull.*, 1978, **26**, 141.
15. G. A. Lane and R. H. Newman, *Phytochemistry*, 1987, **26**, 295.
16. K. G. R. Pachler and W. G. E. Underwood, *Tetrahedron*, 1967, **23**, 1817.
17. M. Afzal and G. Al-Oriquat, *Heterocycles*, 1986, **24**, 2911.
18. a) N. W. Preston, *Phytochemistry*, 1977, **16**, 2044. b) A. Pelter, P. Stainton, and B. Barber, *J. Heterocycl. Chem.*, 1965, **2**, 262.
19. C. M. Brink, J. P. Engelbrecht, and D. E. Graham, *J. South African Chem. Institute*, 1970, **23**, 24.
20. The structures 3 and 9 only indicate the relative configurations.
21. K. S. Dhami and J. B. Stothers, *Can. J. Chem.*, 1966, **44**, 2855.
22. Stability of licoriphenone (4) in isolation must be due to hydrogen-bonding of 2-OH to the carbonyl. It has been demonstrated that the lack of 2-OH group resulted in spontaneous cyclization to the benzofuran derivative, while when 2-OH group is present the compound is stable in phenylacetophenone form as such and sublimed unchanged (W. B. Whalley and G. Lloyd, *J. Chem. Soc.*, 1965, 3213).

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