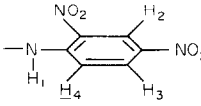
(1H, *d*, $J_{2,3}$ = 7 Hz, H_1–C=C–C–H_3). MS *m/z* 281 (22.9%), 280 $[M]^+$ (86.3), 279 $[M-1]^+$ (11.1), 262 (25.6), 254 (100), 239 (24.8), 236 (13.7), 225 (17.9), 207 (23.1), 181 (24.8), 147 (23.1), 133 (17.9), 115 (38.5), 91 (26.5), 77 (46.2), 55 (70.9), 41 (62.4).

The diacetate of **2** formed a pale yellow syrup; its NMR spectra showed acetoxy groups as singlets (3H) at δ 2.01 and 2.07 which were similar to those of magnolol diacetate, indicating the OH groups in both compounds are in the same positions. Methylation of **2** with CH_2N_2 gave a yellow syrup; its NMR spectra ($CDCl_3$) showed two methoxy groups at δ 3.78 and 3.85. The 2,4-dinitrophenylhydrazones of **2** and its diacetate formed as brown-red needles, mp 232–234° and orange-red needles, mp 198–200°, respectively. The IR and NMR spectra of the latter compound are as follows: IR ν_{max} cm^{-1}: 3300 (NH), 1770, 1750, 1200, 1180 (OCOMe), 1620 (C=N), 1510, 1340 (NO_2), 1010, 920 (monosubstituted alkene), 980 (*trans*-disubstituted alkene). ^1H NMR ($CDCl_3$): δ 2.03 (3H, *s*, $OCOCH_3$), 2.07 (3H, *s*, $OCOCH_3$), 3.43 (2H, *d*, J = 6 Hz, $CH_2CH=CH_2$), 5.10 (2H, *m*, $CH_2CH=CH_2$), 6.03 (1H, *m*, $CH_2CH=CH_2$), 6.97–7.95 (9H, *m*, arom. and HC=CHCH=N–), 7.98 (1H, *d*, $J_{3,4}$ = 9 Hz,



) 8.45 (1H, *dd*, $J_{3,4}$ = 9 Hz, $J_{2,3}$ = 2 Hz, H_3), 9.45 (1H, *d*, $J_{2,3}$ = 2 Hz, H_2), 10.27 (1H, *s*, NH_1). From the above data **2** contains one more alkene and an aldehyde group, but one fewer allyl group than **1**, so the structure for **2** must be 2,2'-dihydroxy-5-allylbiphenyl-5'-propenal.

Isolation of randaiol (**3**), *2,2',5'-trihydroxy-5-allylbiphenyl.* The $CHCl_3$-soluble part of the MeOH extract was chromatographed on a column of Si gel followed by prep. TLC with 30% EtOAc in $CHCl_3$ to afford a yellow syrup, IR ν_{max} cm^{-1}: 3600–3100, 1200 (OH), 1600, 1480, 810 (1,2,4-trisubstituted arom.) 1620, 985, 905 (monosubstituted alkene). ^1H NMR [$(CD_3)CO$]: δ 3.36 (2H, *d*, J = 6 Hz, $CH_2CH=CH_2$), 5.01 (2H, *m*, $CH_2CH=CH_2$), 6.00 (1H, *m*, $CH_2CH=CH_2$), 6.65–7.13 (6H, *m*, arom.), 8.00 (3H, *brs*, 3 × OH). MS *m/z* 242 $[M]^+$ ($C_{15}H_{14}O_3$), i.e. $C_{12}H_6(OH)_3CH_2CH=CH_2$. These data indicated that **3** contains one more OH group but one fewer allyl group than **1**. The triacetate of **3** formed a syrup. ^1H NMR ($CDCl_3$): δ 2.03, 2.04 and 2.23 (each 3H, *s*, $OCOCH_3$), 3.37 (2H, *d*, J = 6 Hz, $CH_2CH=CH_2$), 5.07 (2H, *m*, $CH_2CH=CH_2$), 5.97 (1H, *m*, $CH_2CH=CH_2$), 7.00–7.23 (6H, *m*, arom.). MS *m/z* 368 $[M]^+$ (3.3%), 3.26 $[M-42]^+$ (55), 284 $[M-84]^{+l}$ (63.1), 242 $[M-126]^+$ (100). From the above data the structure of **3** appeared to be 2,2', 5'-trihydroxy-5-allylbiphenyl. Both **2** and **3**, as far as we are aware, are not described in the literature.

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DAPHNETICIN, A COUMARINOLIGNOID FROM *DAPHNE TANGUTICA*

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Key Word Index—*Daphne tangutica*; Thymelaeaceae; daphneticin; coumarinolignoid; 1'-(3,5-dimethoxy-4-hydroxy)phenyl-2'-hydroxymethylethylene-dioxy-1'–O,2'–O:8,7-coumarin.

Abstract—A new coumarinolignoid, daphneticin, has been isolated from the roots of *Daphne tangutica* and its structure elucidated on the basis of chemical and NMR spectroscopic evidence.

INTRODUCTION

Daphne tangutica Maxim (*D. retusa* Hemsl.) has been used in Chinese traditional medicine as an abortifacient and as a remedy for the treatment of rheumatism and toothache. In the course of a screening programme, we have found that a dichlormethane extract of the drug showed a strong *in vitro* inhibition (60%) of the Walker-256-Carcinoma Ascites cells [1]. Fractionation of this extract on a silica gel column with toluene–acetone mixtures yielded four lignans, three coumarins, a diterpene ester [1] and a new coumarinolignoid designated daphneticin. The structure of daphneticin is reported in the present communication.

RESULTS AND DISCUSSION

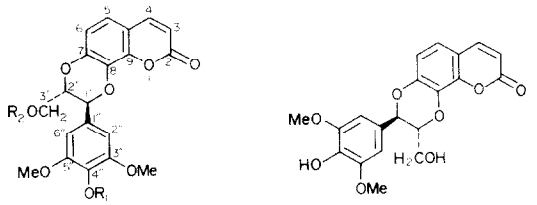
Daphneticin, mp 235–238°, $C_{20}H_{18}O_8$, showed a molecular ion at 386.1006 and had no optical rotation. The UV absorption at 317, 260 and 242 nm, a δ-lactone absorption band at 1730 cm^{-1} in the IR spectrum and two character-

Table 1. ^{13}C NMR spectrum of daphneticin (**1**) and its diacetate (**3**)

	Daphneticin, **1** (C_5D_5N)	Diacetate, **3*** ($CDCl_3$)
C-2	160.4 *s*	160.2 *s*
C-3	113.6 *d*	113.9 *d*
C-4	144.3 *d*	143.8 *d*
C-5	119.8 *d*	119.9 *d*
C-6	113.2 *d*	113.9 *d*
C-7	147.6 *s*	146.5 *s*
C-8	138.4 *s*	133.3 *s*
C-9	149.2 *s*	150.6 *s*
C-10	113.6 *s*	113.7 *s*
C-1″	126.4 *s*	131.7 *s*
C-2″	106.3 *d*	104.0 *d*
C-3″	149.2 *s*	152.8 *s*
C-4″	132.2 *s*	123.3 *s*
C-5″	149.2 *s*	152.8 *s*
C-6″	106.3 *d*	104.0 *d*
C-1′	77.8 *d*	77.2 *d*
C-2′	79.9 *d*	75.5 *d*
C-3′	60.7 *t*	62.7 *t*
–OMe × 2	56.4 *q*	56.3 *q*

*168.5 *s*, 170.2 *s*, 20.5 *q* and 20.6 *q* for two –COMe.

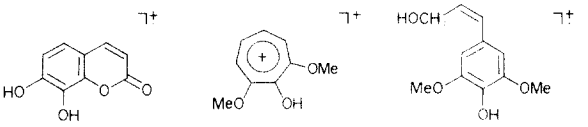
istic doublets (AB pattern) at 6.36 and 8.01 (J = 10 Hz) in connection with a second AB pattern visible at 7.25 and 7.01 (J = 9 Hz) indicated the existence of a coumarin nucleus with a C-7,8-substitution pattern. This substitution type was confirmed by the nearly identical ^1H NMR spectrum in the corresponding region of 7,8-dihydroxycoumarin (daphnetin) isolated from the same plant [1], and the appearance of the daphnetin fragment ion ($C_9H_6O_4$, m/z 178) in the mass spectrum (Fig. 2). According to its formula, daphneticin contains eight oxygen functions with four of them in the $C_{11}H_{14}O_4$ residue (m/z 210), linked to the daphnetin moiety. One of these oxygens occurs as a phenolic group, which was methylated with dimethyl sulphate–potassium carbonate to give a non-phenolic monomethyl ether, **2** ($C_{21}H_{20}O_8$, M^+ 400). Since acetylation of **1** with acetic anhydride–pyridine yielded a diacetate, **3** ($C_{24}H_{22}O_{10}$, M^+ 470), daphneticin also contains an aliphatic hydroxyl group. Further information about the structure of the C_{11}-residue came from the ^1H and ^{13}C NMR spectra of **1**. A singlet at δ6.81 integrating for two aromatic protons together with the presence of two identical methoxy groups indicated a 4-hydroxy-3,5- or 2,6-dimethoxy-



1 $R_1 = R_2 = H$
2 R_1 = Me, R_2 = H
3 $R_1 = R_2$ = Ac

Fig. 1.

*The structure of Cleomiscosin B is that originally proposed for Cleomiscosin A in the first publication [2].



A *m/z* 178 B *m/z* 167 C *m/z* 210.0890

Fig. 2.

substituted benzene ring. A low field chemical shift of $\Delta\delta$ 0.16 (DMSO-d_6) for this singlet after acetylation was in favour of the 4-hydroxy-3,5-dimethoxy-substitution pattern. The ^{13}C NMR spectrum showed 12 aromatic carbons (CH × 4, C × 2, C O × 6), seven aliphatic carbons (Me–O × 2, CH_2–O × 1, CH–O × 2, =CH × 2) and one carbonyl group (Table 1).

The mode of coupling of the daphneticin molecule with the phenylpropane moiety was determined spectroscopically. A retro-Diels–Alder fragmentation of a substituted phenylpropane unit yielded a peak at m/z 210.0890 ($C_{11}H_{14}O_4$) in the mass spectrum. The signals at δ5.10 (a), 4.34 (b) and 3.59 (c) in the ^1H NMR spectrum were found to be consistent with the following bonding sequence: A–CHO′–CHO′–CH_2O'. Since the signal for the CH_2 protons showed a downfield shift of $\Delta\delta$ 0.63 after acetylation, this group must bear the hydroxyl group. The methine group should be connected with the two oxygens of the daphneticin molecule forming a benzodioxan bridge.

The coupling constant between the H-1′ and H-2′ signals in **1** and **3** was 7.6 Hz, demonstrating that the two hydrogens are *trans*-oriented. This coumarinolignoid skeleton is similar to that observed for cleomiscosin A and B, which have been isolated from *Cleome viscosa* [2,3], and cleomiscosin B, recently found in *Simaba multiflora* (Simaroubaceae), *Soulamea soulameoides* (Simaroubaceae) and *Matay'a arborescens* (Sapindaceae) [4]. A third coumarinolignan which has been isolated by Das Graças *et al.* from *Protium opacum* [5] differs from cleomiscosins by a methyl group instead of CH_2OH group on the benzodioxan ring. Bearing one of the two methoxyl groups, in the phenyl ring forming a syringyl residue rather than in the coumarin nucleus on C-6, daphneticin is a structural isomer of cleomiscosin A and B. As for cleomiscosin A and B, it has still to be elucidated which of the two alternative structures (**1** or **4**) is correct.

By analogy with the structure determination of cleomiscosin A and B [3], the decision could be made by measurements of the selective $^{13}C\{^1H\}$heterodecouplings of daphneticin diacetate. Irradiation at the C-2′ hydrogen signal at δ4.40 sharpened the doublet at 146.5 attributed to C-7. The same observation was made for the C-8 signal at δ133.3, when the irradiation took place on the C-1′ hydrogen at 4.98. These spectroscopic results are identical to those observed for cleomiscosin B [3]* and are good evidence for structure **1**. Daphneticin showed cytotoxic activity determined *in vitro* on Walker-256-Carcinosarcoma-Ascites cells [1].

EXPERIMENTAL

Mp on Kofler (corr.); TLC: Si gel HF-254 (Merck); CC: Si gel 60, 0.063 mm (Merck). *Daphne tangutica* stems and roots were collected in June 1980 in Yünnan Province, People's Republic of China. Voucher No. 1005, Herbarium, Institute of Pharmaceutical Biology, München.

Isolation of daphneticin (**1**). 700 g air-dried and powdered roots and stems of *Daphne tangutica* were extracted with CH_2Cl_2 in a Soxhlet for 2 days. The extract was evaporated to a syrup and the residue (20 g) fractionated on a column of Si gel (70 × 7 cm) with toluene–Me_2CO (3:1). The fractionation, monitored by TLC, yielded 10 main fractions [1].

Fraction 9 was rechromatographed on a Si gel column with $CHCl_3$–MeOH (20:1). The fraction containing daphneticin (R_f 0.22 in toluene–Me_2CO, 3:1, R_f 0.32 in $CHCl_3$–MeOH, 20:1, Liebermann–Burchard reagent) was evaporated and the residue crystallized from MeOH–Me_2CO, (0.07 g). Mp 235–238°; $[\alpha]_D^{25}$ = 0° (C_5H_5N; *c* 0.92); $C_{20}H_{18}O_8$ (MS, M^+ 386.1006); UV λ_{max}^{MeOH} nm (ε): 242 (9170), 260 (8892) and 317 (11 230); IR $\bar{\nu}_{max}^{KBr}$ cm^{-1}: 3480, 3210, 1730, 1610, 1570, 1450, 1340, 1270, 1115, 1055, 835; MS *m/z* (rel. int.): 386 (6), 368 (5), 354 (9), 311 (5), 210 (50), 178 (100), 167 (60), 150 (58); ^1H NMR (DMSO-d_6): 8.01, 6.36 (AB pattern, *J* = 10 Hz, H-4, H-3), 7.25, 7.01 (AB pattern, *J* = 9 Hz, H-5, H-6), 6.81 (2H, *s*, H-2″,H-6″), 5.10 (1H, *d*, J = 7.5 Hz, H-1′), 4.34 (1H, *m*, H-2′), 3.81 (6H, *s*,–OMe × 2), 3.59 (2H, *m*, H-3′).

Daphneticin acetate (**3**). 40 mg daphneticin were acetylated with 6 ml C_6H_5N and 6 ml $(MeCO)_2O$ at room temp. for 12 hr and processed in the usual way. Recrystallization from Me_2CO yielded 30 mg **3** mp 208–209°; $C_{24}H_{22}O_{10}$ (MS *m/z* 470, M^+); R_f 0.5 ($CHCl_3$–Me_2CO, 10:1); IR $\bar{\nu}_{max}^{KBr}$ cm^{-1}: 1730, 1610, 1570, 1450, 1360, 1200, 1055, 835; ^1H NMR (DMSO-d_6): 8.02, 6.38 (AB pattern, *J* = 10 Hz, H-4, H-3), 7.28, 7.04 (AB pattern, *J* = 9 Hz, H-5, H-6), 6.97 (2H, *s*, H-2″, H-6″), 5.25 (1H, *d*, *J* = 7.5 Hz, H-1′), 4.79 (1H, *m*, H-2′), 4.22 (2H, *m*, H-3′), 3.83 (6H, *s*, –OMe × 2), 2.31 (3H, *s*, Ar–OAc), 2.09 (3H, *s*, $-CH_2$–OAc); 90 MHz, in $CDCl_3$: 7.67, 6.33 (AB pattern, *J* = 10 Hz, H-4, H-3), 7.02, 6.93 (AB pattern, *J* = 9 Hz, H-5, H-6), 6.62 (2H, *s*, H-2″, H-6″), 4.98 (1H, *d*, *J* = 7.5 Hz, H-1′), 4.40 (1H, *m*, H-2′), 4.18 (2H, *m*, H-3′), 3.84 (6H, *s*, –OMe × 2), 2.33 (3H, *s*, Ar–OAc), 2.07 (3H, *s*, aliph. OAc).

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ASTILBIN AND ENGELETIN IN GRAPES AND WINE

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Key Word Index—*Vitis vinifera*; Vitaceae; grapes; dihydroquercetin 3-rhamnoside; dihydrokaempferol 3-rhamnoside; flavanonols; wines.

Abstract—Astilbin (dihydroquercetin 3-rhamnoside) and engeletin (dihydrokaempferol 3-rhamnoside) were isolated for the first time from grapes. Details of their identification include nonderivatized ^1H NMR spectra. These flavanonol glycosides were concentrated in the skins of white grapes, and were also present in white wines as shown by HPLC. Amounts and relative amounts differed by cultivar. They may be involved in certain discoloration problems during wine processing.

INTRODUCTION

A case can be made that the grape and the products made from it constitute the most important fruit crop of the world [1, 2]. Furthermore, phenols are not only important factors in characterizing the different grapes and grape products, but also through these products enter the human diet in large total amounts. For these reasons and because different natural phenols can have quite different roles in browning and other discoloration reactions [3], flavor contribution [4] and oxidative reactions in foods including wines, considerable interest attaches to the qualitative and quantitative phenol composition of grapes.

In the course of HPLC studies of white grapes and their wines, two phenols not previously reported from grapes or wines were isolated and identified as the flavanonol rhamnosides astilbin and engeletin. While these compounds are known from a few other plant sources, none of these is important as foods. In fact, dihydroflavonols