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THE NEOLIGNANS, CARINATIDIN, DIHYDROCARINATIDIN, CARINATIDIOL AND DEHYDRODIEUGENOL B FROM *VIROLA CARINATA*

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Key Word Index—*Virola carinata*; Myristicaceae; bark; neolignans; carinatidin; dihydrocarinatidin; carinatidiol; dehydrodieugenol B.

Abstract—Seven neolignans have been isolated from the bark of *Virola carinata*. Four more neolignans were found to be 5-allyl-7-methoxy-3-methyl-2-(3′-methoxy-4′-hydroxyphenyl)benzofuran, (2*S*,3*S*)-5-allyl-7-methoxy-3-methyl-2-(3′-methoxy-4′-hydroxyphenyl)dihydrobenzofuran, *threo*-1-(3′,4′-dimethoxyphenyl)-2-(2″-methoxy-4″-allylphenoxy) propane diol (1, 3) and 4,5′-diallyl-2′-hydroxy-2,3′-dimethoxy diphenyl ether. The first three compounds were newly isolated and called carinatidin, dihydrocarinatidin and carinatidiol, respectively. The last one, known as dehydrodieugenol B, has been isolated before from *Ocotea cymbarum*.

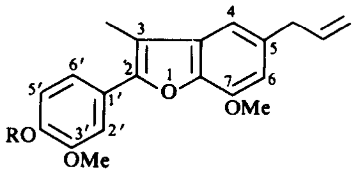
INTRODUCTION

In our previous three reports on *Virola carinata* (Benth) Warburg, seven neolignans were determined as dehydrodieugenol [1], its monomethyl ether [1], carinatone [2], carinatin [2], dihydrocarinatinol [3], carinatonol [3] and carinatol [3]. The further isolation of neolignans was continued and the determination of the structures of four additional compounds is described in the present paper.

RESULTS AND DISCUSSION

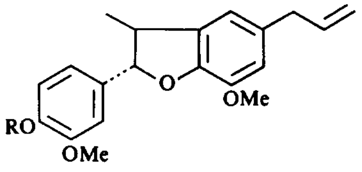
The benzene fraction [1] was chromatographed several times to separate four more neolignans besides carinatin and carinatone. The molecular formula, $C_{20}H_{20}O_4$ of compound 1 (needles mp 104–105°) was based on high resolution mass spectrometry. It was a phenolic compound, giving a blue colour with ferric chloride–potassium ferricyanide. Compound 1 was very similar to carinatin (**2**) [2] on UV and IR. Its ^{1}H NMR showed two methoxyl and a hydroxyl group substituted on a benzene and a benzofuran ring, whereas carinatin has three methoxyl groups on such rings. Methylation of compound 1 with diazomethane gave a methyl ether, which was identical with carinatin (**2**) by means of mmp, IR, ^{1}H NMR and mass spectra. Compound 1 was oxidized by hydrogen peroxide [4] to obtain vanillic acid, which was shown to be identical to an authentic sample by means of mmp, IR, ^{1}H NMR and mass spectra. The hydroxyl group was located on C-4′ of the benzene ring. Therefore compound 1 (**1**) was determined to be 5-allyl-7-methoxy-3-methyl-2-(3′-methoxy-4′-hydroxyphenyl)benzofuran and named carinatidin.

Compound 2, a resin, $C_{20}H_{22}O_4$ estimated by high resolution mass spectrometry, was also a phenol derivative giving a positive colour reaction with ferric chloride–potassium ferricyanide and a hydroxyl absorbance by IR. It resembled dihydrocarinatin (**4**) [2] on UV and monodemethyl dihydrocarinatin on ^{1}H NMR. Compound 2 was methylated with dimethylsulfate and dehydrogenated with palladium–charcoal to give dihydrocarinatin (**4**) and compound 1, respectively, which were confirmed by means of TLC, IR and mass spectra. The configuration of compound 2 was shown to be 2*S* and 3*S* by comparison with dihydrocarnatin (2*S*, 3*S*) (**4**) [2]; optical rotation ($[\alpha]_D$ − 12.7°) and AMX_3 system for 3-methyl dihydrobenzofuran derivatives (2*S*,3*S*) on ^{1}H NMR [2]: methine proton (A) on C-2 at $\delta 5.10$ (doublet, $J = 10.0$ Hz), methine proton (M) on C-3 at $\delta 3.45$ (doublet quartet, $J = 10.0$ and 6.7 Hz) and methyl protons (X_3) on C-3 at $\delta 1.37$ (doublet, $J = 6.7$ Hz). Thus compound 2 (**3**) was identified as (2*S*,3*S*)-5-allyl-7-methoxy-3-methyl-2-(3′-methoxy-4′-hydroxyphenyl)dihydrobenzofuran and named dihydrocarinatidin.



1 R=H

2 R=Me



3 R=H

4 R=Me

Compound 3 was separated as a resin, $C_{21}H_{26}O_6$ and estimated by high resolution mass spectrometry. An absorbance of ν3500 cm^{-1} on IR indicated the presence of an alcoholic hydroxy group, since it gave a negative phenol test with ferric chloride–potassium ferricyanide. ^{1}H NMR spectral measurements using decoupling techniques suggested that compound 3 was a derivative of veratryl glycerol possessing a 2-methoxy 4-allyl phenyl group: δ3.87, 3.88 and 3.90 for three methoxyl groups, δ3.36, 5.09, 5.11 and 5.97 and α-methylene, γ-methine (*cis*), γ-methine (*trans*) and β-methine of allyl group, respectively, δ3.48, 3.63, 3.98 and 4.99 for methylene protons at C-3 and methines at C-2 and C-1 of the glycerol moiety, respectively, and δ6.77–7.06 for six aromatic protons. Guaiacyl glycerol derivatives and their glycosides have been reported from *Thuja plicata* [5], *Pinus sylvestris* [6] and *Picea abies* [7]. Acetylation of compound 3 yielded the diacetate (**6**), which was proved by the detection of carbonyl absorbance at ν1740 cm^{-1} on IR, two singlet acetyl protons at δ2.01 and 2.03 on ^{1}H NMR and the $[M]^+$ at *m/z* 458 ($C_{25}H_{30}O_8$) by mass spectrometry. Two acetyl groups on C-3 and C-1 of the glycerol moiety of the diacetate (**6**) lower the two hydrogen signals on C-3 and one on C-1 to δ4.99, 4.28 and 6.08, respectively. The actual decrease of the proton signal on C-1 of the glycerol moiety for the diacetate (**6**) comparing with its parent compound 3 (**5**) was 1.09 ppm, which approximated to the decreases; 1.03 [8] and 1.14 [6] for 1-(3′,4′-dimethoxyphenyl)-2-(2″-methoxyphenoxy)-propane diol (1, 3) diacetate and 1-(4′-hydroxy-3′-methoxyphenyl)-2-(4″-(3-hydroxypropyl)-2″-hydroxyphenoxy) propane diol (1, 3) diacetate with their parent compounds, respectively. ^{13}C NMR spectrum also supported that compound 3 was a glycerol derivative; δ60.99 (triplet), 89.74 (doublet) and 73.93 (doublet) for C-3, C-2 and C-1 on the glycerol moiety, respectively. The structure of compound 3 was verified by high resolution mass spectra and those of its acetate (**6**) (Scheme 1). On further analysis of the ^{1}H NMR of compound 3, the coupling constant (J = 8.2 Hz) observed between the two protons on the C-1 and C-2 of the glycerol moiety indicated that compound 3 existed as the *threo* isomer [6]. Thus compound 3 (**5**) was determined as *threo*-1-(3′,4′-dimethoxyphenyl)-2-(2″-methoxy-4″-allylphenoxy) propane diol (1, 3) and named carinatidiol.

Compound 4, a resin, $C_{20}H_{22}O_4$ and its acetate $C_{22}H_{24}O_5$ estimated by high resolution mass spectrometry were identical to dehydrodieugenol B, isolated from *Ocotea cymbarum* and its acetate on ^{1}H NMR and mass spectrometry, respectively.

Eleven neolignans have now been isolated from the bark of *Virola carinata*. Three of them; carinatinol [3], carinatonol [3] and carinatidiol were exceedingly rare to be mixed oxidative C_6–C_3 dimers (one oxygenated and one unoxygenated γ-carbon), while furoguaiacidin [10] was the first one reported as a natural plant constituent.

EXPERIMENTAL

Isolation of compounds. On silica gel CC of a C_6H_6 extract [1], C_6H_6–Me_2CO (49:1) gave two fractions. The first one contained carinatin and carinatone, and the second one was a mixture of compounds 1, 2 and 4. C_6H_6–Me_2CO (17:3) eluted compound 3, which was purified by further CC with EtOAc–C_6H_6–*iso*PrOH (3:3:0.4). Compound 1 and a mixture of compounds 2 and 4 were eluted with petrol–Me_2CO (92.5:7.5) and (9:1) on additional CC. Compound 2 was separated from compound 4 by prep. TLC using C_6H_6–Me_2CO (20:1) on silica gel.

Compound 1. Colourless needles, mp 104–105°, bluish green colour with 1 N $FeCl_3$–1 N $K_3Fe(CN)_6$, $C_{20}H_{20}O_4$ (found 324.134, requires 324.136). UV $\lambda_{\max}^{\mathrm{CHCl_3}}$ nm (log ε): 307 (4.59), 243 (4.33). IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3560, 3015, 2945, 2845, 1625, 1600, 1520, 1493, 1468, 1397, 1388, 1308, 1265, 1142, 1058, 1025, 917, 880, 808. ^{1}H NMR (200 MHz, $CDCl_3$): δ2.40 (3H, *s*, Me-3), 3.50 (2H, *dt*, J = 6.8 and 1.6 Hz, $\mathrm{C\underline{H}_2{-}CH{=}CH_2}$), 4.00 (3H, *s*, OMe), 4.04 (3H, *s*, OMe), 5.09 (1H, *ddt*, J = 10.0, 2.0 and 1.6 Hz, $\mathrm{CH_2{-}CH{=}C\underline{H}_2}$ (*cis*)), 5.14 (1H, *ddt*, J = 17.0, 2.0 and 1.6 Hz, $\mathrm{CH_2{-}CH{=}C\underline{H}_2}$ (*trans*)), 5.76 (1H, *s*, OH), 6.06 (1H, *ddt*, J = 17.0, 10.0 and 6.8 Hz, $\mathrm{CH_2{-}C\underline{H}{=}CH_2}$), 6.66 (1H, *d*, J = 1.2 Hz, H-6), 6.95 (1H, *d*, J = 1.2 Hz, H-4), 7.02 (1H, *d*, J = 8.0 Hz, H-5′), 7.32 (1H, *dd*; J = 8.0 and 2.0 Hz, H-6′), 7.35 (1H, *d*, J = 2.0 Hz, H-2′). MS *m/z* (rel. int.): 324 $[M]^+$ (47.3), 83 (100.0).

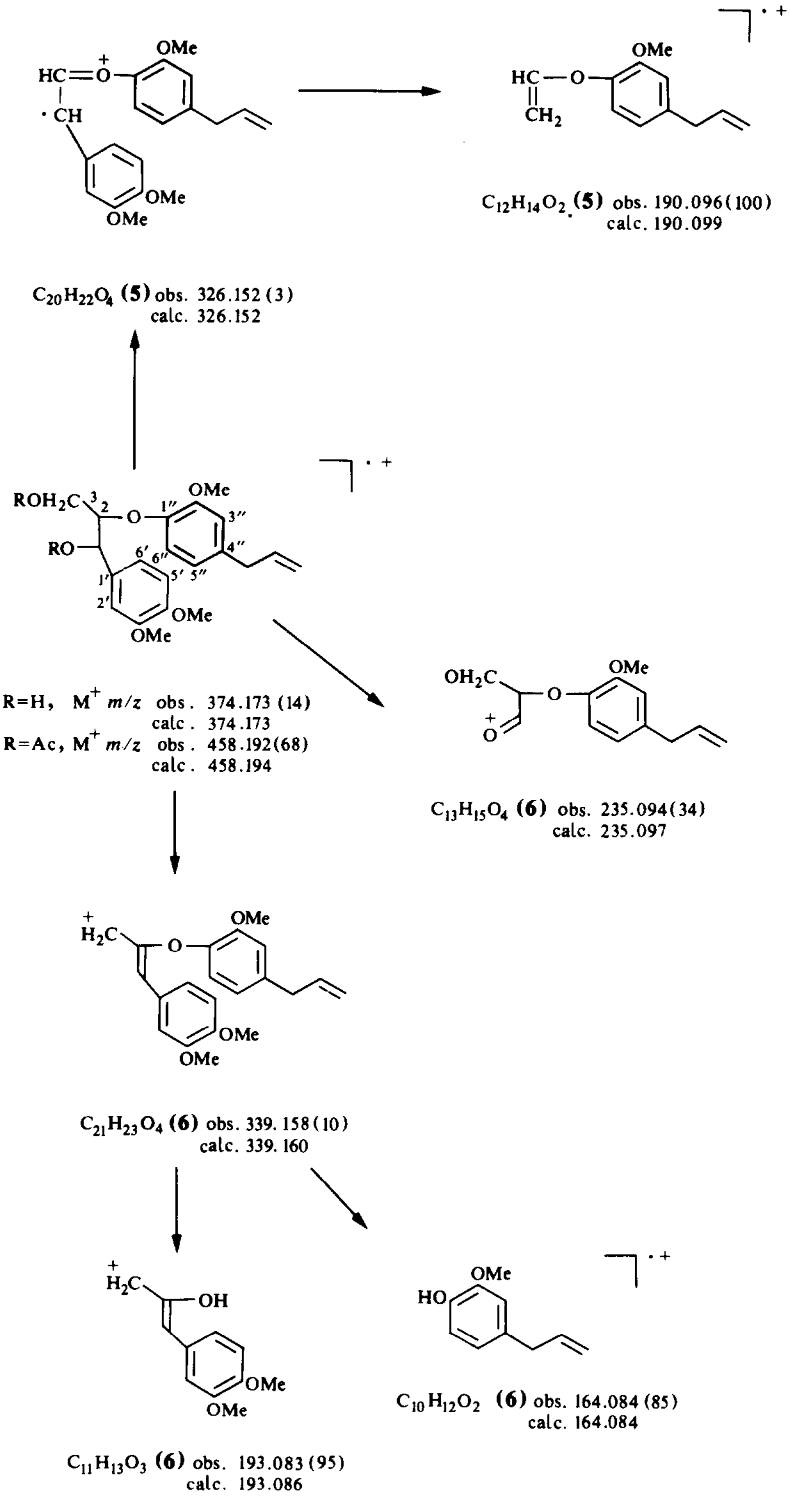
Compound 2. A resinous mass, bluish green colour with 1 N $FeCl_3$–1 N $K_3Fe(CN)_6$, $C_{20}H_{22}O_4$ (found 326.153, requires 326.153). $[\alpha]_D$ −12.7° ($CHCl_3$). UV $\lambda_{\max}^{\mathrm{CHCl_3}}$ nm (log ε): 285 (3.82), 282 (3.83), 244.5 (3.93). IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3700, 2930, 2855, 1600, 1500, 1465, 1270, 1135. ^{1}H NMR ($CDCl_3$): δ1.37 (3H, *d*, J = 6.7 Hz, Me-3), 3.37 (2H, *dt*, J = 6.7 and 1.6 Hz, $\mathrm{C\underline{H}_2{-}CH{=}CH_2}$), 3.45 (1H, *dq*, J = 10.0 and 6.7 Hz, H-3), 3.87 (3H, *s*, OMe), 3.88 (3H, *s*, OMe), 5.08 (1H, *ddt*, J = 10.2, 2.0 and 1.6 Hz, $\mathrm{CH_2{-}CH{=}C\underline{H}_2}$ (*cis*)), 5.10 (1H, *d*, J = 10.0 Hz, H-2), 5.13 (1H, *ddt*, J = 17.0, 2.0 and 1.6 Hz, $\mathrm{CH_2{-}CH{=}C\underline{H}_2}$ (*trans*)), 5.61 (1H, *s*, $\mathrm{O\underline{H}}$), 6.00 (1H, *ddt*, J = 17.0, 10.2 and 6.7 Hz, $\mathrm{CH_2{-}C\underline{H}{=}CH_2}$), 6.62 (1H, *br s*, H-4 or H-6), 6.65 (1H, *br s*, H-6 or H-4), 6.92 (2H, *br s*, H-5′ and H-6′), 7.01 (1H, *br s*, H-2′). MS *m/z* 326 $[M]^+$ (100.0), 149.(28.3).

Compound 3. A resinous mass, no colour with 1 N $FeCl_3$–1 N $K_3Fe(CN)_6$, $C_{21}H_{26}O_6$ (found 374.173, requires 374.173). $[\alpha]_D$ +97.2° ($CHCl_3$). UV $\lambda_{\max}^{\mathrm{CHCl_3}}$ nm (log ε): 285.5 (4.04), 248.5 (4.09). IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3500, 3010, 2945, 2840, 1590, 1515, 1465, 1265, 1140, 1025, 920. ^{1}H NMR ($CDCl_3$): δ3.36 (2H, *dt*, J = 7.0 and 1.2 Hz, $\mathrm{C\underline{H}_2{-}CH{=}CH_2}$), 3.48 (1H, *dd*, J = 12.5 and 3.3 Hz, H-3), 3.63 (1H, *dd*, J = 12.5 and 3.3 Hz, H-3), 3.87 (3H, *s*, OMe), 3.88 (3H, *s*, OMe), 3.90 (3H, *s*, OMe), 3.98 (1H, *dt*, J = 8.2 and 3.3 Hz, H-2), 4.99 (1H, *d*, J = 8.2 Hz, H-1), 5.09 (1H, *ddt*, J = 10.0, 2.0 and 1.2 Hz, $\mathrm{CH_2{-}CH{=}C\underline{H}_2}$ (*cis*)), 5.11 (1H, *ddt*, J = 18.0, 2.0 and 1.2 Hz, $\mathrm{CH_2{-}CH{=}C\underline{H}_2}$ (*trans*)), 5.97 (1H, *ddt*, J = 18.0, 10.0 and 7.0 Hz, $\mathrm{CH_2{-}C\underline{H}{=}CH_2}$), 6.77 (1H, *dd*, J = 7.0 and 2.0 Hz, H-5″), 6.86 (1H, *dd*, J = 8.7 and 3.0 Hz, H-6′), 6.91 (1H, *d*, J = 7.0 Hz, H-6″), 6.98 (1H, *d*, J = 2.0 Hz, H-3″), 7.01 (1H, *d*, J = 3.0 Hz, H-2′), 7.06 (1H, *d*, J = 8.7 Hz, H-5′). ^{13}C NMR (50 MHz, $CDCl_3$): δ39.97 (*t*, $\mathrm{C\underline{H}_2{-}CH{=}CH_2}$), 55.91 (*q*, OMe × 3), 60.99 (*t*, C-3), 73.93 (*d*, C-1), 89.74 (*d*, C-2), 109.87 (*d*, C-2′), 110.99 (*d*, C-5′), 112.46 (*d*, C-3″), 116.08 (*t*, $\mathrm{CH_2{-}CH{=}\underline{C}H_2}$), 119.65 (*d*, C-6′), 121.07 (*d*, C-6″), 121.54 (*d*, C-5″), 132.11 (*s*, C-1′), 136.46 (*s*, C-4″), 137.17 (*d*, $\mathrm{CH_2{-}\underline{C}H{=}CH_2}$), 145.79 (*s*, C-2″), 148.89 (*s*, C-4′), 149.09 (*s*, C-3′), 151.17 (*s*, C-1″). MS *m/z* 374 $[M]^+$ (14.0), 326 (2.7), 190 (100.0).

Compound 4. A resinous mass, blue colour with 1 N $FeCl_3$–1 N $K_3Fe(CN)_6$, $C_{20}H_{22}O_4$ (326.150, requires 326.151).

Methylation of compound 1. Treatment of compound 1 with CH_2N_2 gave a Me ether, identical with carinatin by means of mmp, IR, ^{1}H NMR and MS.

Oxidation of compound 1. Compound 1 (20 mg), HOAc (0.15 ml) and H_2O_2 (0.015 ml) were heated at 60° for 8 hr and cooled. To the reaction mixture, a small quantity of H_2O was added and then the solvent was removed *in vacuo*. 2 N KOH (0.4 ml) was added to the residue, the soln refluxed at 100° for 2 hr, cooled and extracted with EtOAc. After concn, the EtOAc extract was acidified with HCl, extracted with EtOAc, dried (Na_2SO_4), concd and purified by prep. TLC to obtain vanillic acid, which was identical to an authentic sample (mmp, IR, ^{1}H NMR and MS).



Scheme 1. Interpreted mass spectrum of compound 3 (**5**) and its acetate (**6**).

Methylation of compound 2. Reaction of compound **2** with Me_2SO_4 and K_2CO_3 in Me_2CO gave a Me ether, identical to dihydrocarinatin (IR, ^{1}H NMR and MS).

Dehydrogenation of compound 2. Compound 2 (50 mg) in 5 ml decalin with Pd–C (10%, 3 mg) was refluxed for 24 hr. The product was chromatographed on silica gel with *n*-hexane and C_6H_6, crystallized and found to be identical with compound 1 (mmp, IR and MS).

Acetylation of compound 3. Prepared in the usual way, a resinous mass, $C_{25}H_{30}O_8$ (458.192, requires 458.194).

IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3010, 2945, 2845, 1740, 1595, 1515, 1465, 1375, 1270, 1140, 1025, 920. ^{1}H NMR ($CDCl_3$): δ2.01 (3H, *s*, OAc), 2.03 (3H, *s*, OAc), 3.22 (2H, *dt*, $J = 6.8$ and 1.2 Hz, $C\underline{H}_2$–$CH{=}CH_2$), 3.81 (3H, *s*, OMe), 3.86 (6H, *s*, OMe × 2), 3.99 (1H, *dd*, $J = 12.0$ and 6.0 Hz, H-3), 4.28 (1H, *dd*, $J = 12.0$ and 4.0 Hz, H-3), 4.58 (1H, *ddd*, $J = 6.8$, 6.0 and 4.0 Hz, H-2), 5.07 (1H, *ddt*, $J = 11.0$, 2.0 and 1.2 Hz, CH_2–$CH{=}C\underline{H}_2$ (*cis*)), 5.08 (1H, *ddt*, $J = 17.0$, 2.0 and 1.2 Hz, CH_2–$CH{=}C\underline{H}_2$ (*trans*)), 5.96 (1H, *ddt*, $J = 17.0$, 11.0 and 6.8 Hz, CH_2–$C\underline{H}{=}CH_2$), 6.08 (1H, *d*, $J = 6.8$, H-1), 6.70 (1H, *dd*, $J = 8.2$ and 2.5 Hz, H-5″), 6.71 (1H, *d*, $J = 2.0$ Hz, H-2′), 6.84 (1H, *d*, $J = 8.2$ Hz, H-5′), 6.91 (1H, *d*, $J = 8.2$ Hz, H-6″), 6.93 (1H, *d*, $J = 2.5$ Hz, H-3″), 6.97 (1H, *dd*, $J = 8.2$ and 2.0 Hz, H-6′). MS *m/z* 458 $[M]^+$ (68.4), 339 (9.7), 235 (34.1), 193 (95.0), 164 (84.9), 43 (100.0).

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