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BENZOYL ESTERS AND AMIDES, STYRYLPYRONES AND NEOLIGNANS FROM THE FRUITS OF *ANIBA RIPARIA**

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Key Word Index—*Aniba riparia*; Lauraceae; benzylbenzoates; *N*-benzoyltyramines; styrylpyrones; neolignans; biodynamic properties.

Abstract—The fruits of *Aniba riparia* contain a series of benzyl esters and phenylethyl amides of benzoic acids, accompanied by styrylpyrones and neolignans. This is the first report on the co-occurrence of representatives of the two latter classes of natural products in the same species.

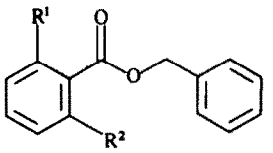
INTRODUCTION

Previous work on trunk wood of *Aniba riparia* (Nees) Mez (Lauraceae) revealed the presence of several flavonoids, benzyl benzoate and benzaldehyde [2, 3]. The present work refers to the unripe fruits of the same species. Twenty five compounds were isolated, three benzylbenzoates (**1a–1c**), six phenylethyl amides of benzoic acids (**2a–2f**), three styryl-α-pyrones (**3a–3c**), two neolignans of the hexahydrobenzofuran type (**4a, 4b**), four neolignans of the tetrahydrobenzofuran type (**5** and the rearrangement products **6a–6c**), one neolignan of the dihydrobenzofuran type (the rearrangement product **7**), as well as 4α-hydroxyeudesem-7(11)-ene (**8**), *O*-methyltyramine (**9**), 2,6-dihydroxybenzoic acid (**10**), mioinositol (**11**), sitosterol (**12**) and epicatechin (**13**), isolated as pentaacetate after acetylation of a chromatographic fraction.

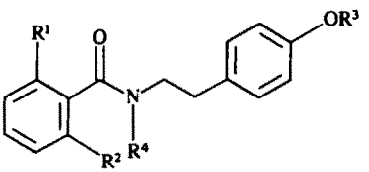
RESULTS AND DISCUSSION

Benzylbenzoate (**1a**) and benzylsalicylate (**1b**) are common constituents of *Aniba* species [4]. In contrast, benzyl-2,6-dihydroxybenzoate (**1c**) has so far been isolated only from two sources, *Aniba kappleri* [5] and *Uvaria purpurea* [6]. All three compounds were identified by spectral means. The structure of **1c** was confirmed by hydrolysis to the expected acid and alcohol, as well as by the traditional synthetic esterification reaction [7] involving 2,6-dihydroxybenzoyl chloride and benzyl alcohol.

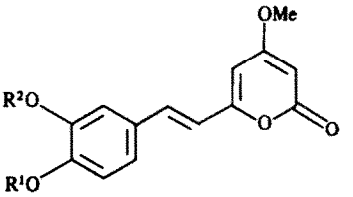
N-Benzoyltyramine (**2a**) and its methyl ether (**2b**) are known compounds having been isolated previously from Rutaceae [8]. In contrast **2c–2f** have not been previously described. The structural elucidations relied on comparison of their spectra with the spectra of the known amides. Hydrolysis to the expected acids and amines, as well as, in the case of **2e** the preparation of derivatives (**2g**,



1a $R^1 = R^2 = H$
1b $R^1 = OH$, $R^2 = H$
1c $R^1 = R^2 = OH$

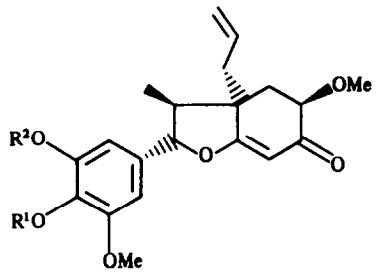


2a $R^1 = R^2 = R^3 = R^4 = H$
2b $R^1 = R^2 = R^4 = H$, $R^3 = Me$
2c $R^1 = OH$, $R^2 = R^3 = R^4 = H$
2d $R^1 = OH$, $R^2 = R^4 = H$, $R^3 = Me$
2e $R^1 = R^2 = OH$, $R^3 = Me$, $R^4 = H$
2f $R^1 = OH$, $R^2 = OMe$, $R^3 = Me$, $R^4 = H$
2g $R^1 = R^2 = OAc$, $R^3 = Me$, $R^4 = H$
2h $R^1 = R^2 = OAc$, $R^3 = Me$, $R^4 = Ac$

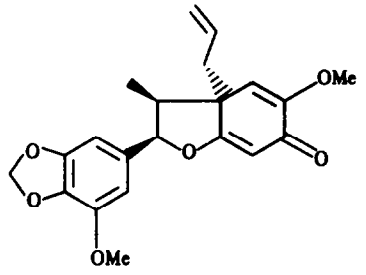


3a $R^1 = H$, $R^2 = Me$
3b $R^1 = R^2 = Me$
3c $R^1—R^2 = CH_2$

*Part LXXXV in the series 'The Chemistry of Brazilian Lauraceae', for Part LXXXIV see ref. [1]. Based on the Doctorate thesis presented by J.M.B.-F. to Universidade de São Paulo (1986).



4a R^1—R^2 = CH_2
4b R^1 = R^2 = Me



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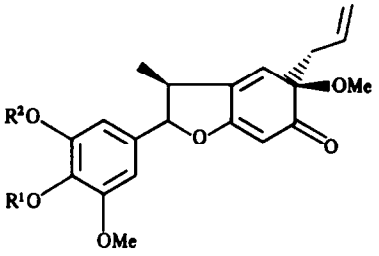
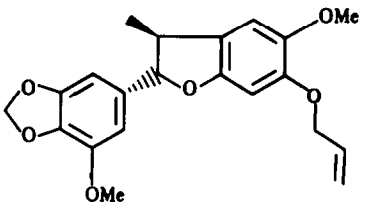


6a R^1—R^2 = CH_2, β-Ar
6b R^1—R^2 = CH_2, α-Ar
6c R^1 = R^2 = Me, α-Ar



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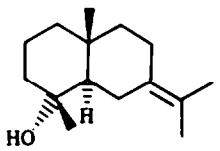

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2h) and syntheses involving the required benzoylchlorides and phenylethylamines, confirmed the identifications. *O*-Methyltyramine (**9**) and 2,6-dihydroxybenzoic acid (**10**) were also found in the free state in the plant extract.

Styrylpyrones such as **3b** and **3c** have been located previously in several *Aniba* species [4]. In contrast, the 4-hydroxy-3-methoxy derivative **3a** has not been isolated previously from Lauraceae, but from Piperaceae [9]. Its identification relied initially on spectral data and then conclusively on its methylation to **3b**. The neolignans **4a** [10], **5**, **6b**, **6c** [11], **6a** and **7** [12] were isolated previously from other *Aniba* species. The new compound **4b** was identified by comparison of its spectral and chiroptical data with the analogous data of **4a**.

Styrylpyrones and neolignans, though common constituents of Lauraceae, have so far never been found together in *Aniba* species [13]. True, previously only bark and wood have been examined. The present report refers for the first time to the chemical composition of the fruits of an *Aniba* species where, again for the first time, styrylpyrones and neolignans were both encountered. The phenylethyl amides of benzoic acids were tested *in vitro* for antibiotic properties. Compound **2e** proved to be active against all the selected microorganisms. Indeed it was shown to be the sole amide active against yeast. All amides, with the exception of **2c**, caused inhibition zones in the bioautographic assay and proved to be active against at least one of the fungi of the genus *Cladosporium*.

EXPERIMENTAL

Isolation of the constituents. Green fruits of *A. riparia* (botanical material identified by Prof. Klaus Kubitzki, Hamburg University) were collected by Dr Hipolito F. Paulino-Filho (Universidade Estadual Paulista 'Julio de Mesquita Filho') in the vicinity of Humaitá, Amazonas State, Brazil. The fruits (5.0 kg) were ground and extracted with EtOH (room temp.). The filtered solution was evapd. The residue (380 g) was redissolved in 60% aq. EtOH. The soln was extracted first with hexane and next with $CHCl_3$. The solvents were evapd and the hexane extract (88 g) was cryst. from MeOH giving triglycerides (79 g). The mother liquor was evapd and the residue was submitted to CC (silica gel). Elution with solvent of increasing polarity gave in order **1a** (895 mg), **1b** (210 mg), **8** (120 mg) and **12** (75 mg). The $CHCl_3$ extract (59 g) was cryst. from C_6H_6 giving **2e** (17 g). The mother liquor was evapd and the residue treated in the same way giving in order **2e** (3 g), **2f** (198 mg), **6b** (20 mg), **4a** (3 mg), **5** (5 mg), **1c** (135 mg), **2b** (305 mg), **2d** (427 mg), **3c** (8 mg), **3b** (315 mg), **6a** (7 mg), **6c** (9 mg), **4b** (10 mg), **7** (10 mg), **2a** (162 mg), **2c** (128 mg), **3a** (1 g), **13** (25 mg), **10** (280 mg) and **9** (305 mg). Upon addition

of Me_2CO to the hydro-alcoholic solution, previously extracted with hexane and $CHCl_3$, **11** (219 mg) precipitated and was collected by filtration.

Identifications of **8** [14], **9**, **10**, **11**, **12** and **13** were done by spectral means.

N-[8′-(4′-*Hydroxyphenylethyl*)]-2-*hydroxybenzoylamide* (**2c**). Mp 135–137° (ether). UV λ_{max}^{MeOH} nm: 225, 240, 305 (ε 5650, 4000, 1650). IR ν_{max}^{KBr} cm^{-1}: 3420, 1635. ^1H NMR [60 MHz, $(CD_3)_2CO$]: δ 14.00 (*br s*, OH), 8.32 (*br s*, NH), 7.95–6.75 (*m*, H-3, H-4, H-5, H-6), 7.22 (*d*, *J* = 8 Hz, H-2′, H-6′), 6.82 (*d*, *J* = 8 Hz, H-3′, H-5′), 3.62 (*br t*, *J* = 7 Hz, 2H-8′), 2.88 (*t*, *J* = 7 Hz, 2 H-7′). ^{13}C NMR (20 MHz, $CDCl_3$): δ 170.9 (*s*, C-7), 162.5 (*s*, C-2), 156.8 (*s*, C-4′), 134.5 (*d*, C-4), 131.0 (*s*, C-1′), 130.5 (*d*, C-2′, C-6′), 127.4 (*d*, C-6), 119.2 (*d*, C-5), 118.6 (*d*, C-3), 116.1 (*d*, C-3′, C-5′), 115.6 (*s*, C-1), 42.1 (*t*, C-8′), 35.3 (*t*, C-7′). MS *m/z* (rel. int.): $[M]^+$ 257 (8), 120 (100), 107 (22).

N-[8′-(4′-*Methoxyphenylethyl*)]-2-*hydroxybenzolylamide* (**2d**). Mp 111–112° (C_6H_6). UV λ_{max}^{MeOH} nm: 225, 240, 305 (ε 5850, 3500, 1650). IR ν_{max}^{KBr} cm^{-1}: 3400, 1650. ^1H NMR (60 MHz, $CDCl_3$): δ 12.48 (*s*, OH), 7.60–6.68 (*m*, H-3, H-4, H-5, H-6), 7.38 (*d*, *J* = 8 Hz, H-2′, H-6′), 6.98 (*d*, *J* = 8 Hz, H-3′, H-5′), 6.45 (*br s*, NH), 3.80 (*s*, OMe), 3.65 (*br t*, *J* = 7 Hz, 2H-8′), 2.86 (*t*, *J* = 7 Hz, 2H-7′). ^{13}C NMR (20 MHz, $CDCl_3$): δ 170.0 (*s*, C-7), 162.0 (*s*, C-2), 158.5 (*s*, C-4′), 134.1 (*d*, C-4), 130.5 (*s*, C-1′), 129.7 (*d*, C-2′, C-6′), 125.4 (*d*, C-6), 118.6 (*d*, C-3, C-5), 114.4 (*s*, C-1, *d*, C-3′, C-5′), 55.3 (*q*, OMe), 41.0 (*t*, C-8′), 35.0 (*t*, C-7′). MS *m/z* (rel. int.): $[M]^+$ 271 (0.1), 121 (81), 134 (100), 121 (81).

N-[8′-(4′-*Methoxyphenylethyl*)]-2,6-*dihydroxybenzoylamide* (**2e**). Mp 131–133° (C_6H_6). UV λ_{max}^{MeOH} nm: 220, 255, 312 (ε 14350, 6300, 2150). IR ν_{max}^{KBr} cm^{-1}: 3360, 1645. ^1H NMR (60 MHz, $CDCl_3$): δ 14.5 (*br s*, OH), 8.92 (*br s*, NH), 7.20 (*t*, *J* = 8 Hz, H-4), 7.18 (*d*, *J* = 8 Hz, H-2′, H-6′), 6.85 (*d*, *J* = 8 Hz, H-3′, H-5′), 6.50 (*d*, *J* = 8 Hz, H-3, H-5), 3.69 (*s*, OMe), 3.63 (*br t*, J = 7 Hz, 2H-8′), 2.85 (*t*, *J* = 7 Hz, 2H-7′). ^{13}C NMR (20 MHz, $CDCl_3$): δ 170.6 (*s*, C-7), 160.0 (*s*, C-2, C-6), 158.4 (*s*, C-4′), 133.4 (*d*, C-4), 131.0 (*s*, C-1′), 129.8 (*d*, C-2′, C-6′), 114.2 (*d*, C-3′, C-5′), 108.2 (*d*, C-3, C-5), 103.3 (*s*, C-1), 55.3 (*q*, OMe), 41.0 (*t*, C-8′), 34.7 (*t*, C-7′). MS *m/z* (rel. int.): $[M]^+$ 287 (14), 137 (92), 134 (100), 121 (37). Acetylation of **2e** (100 mg, Ac_2O 2.5 ml, C_5H_5N 2.5 ml, 3 hr under reflux) gave a mixture of **2g** (35 mg) and **2h** (60 mg), which was separated by TLC. *Diacetate* (**2g**), mp 108–110° (Me_2CO–hexane). IR ν_{max}^{KBr} cm^{-1}: 3400, 1765, 1650. ^1H NMR (60 MHz, $CDCl_3$): δ 7.65–7.00 (*m*, H-3, H-4, H-5), 7.25 (*d*, *J* = 8 Hz, H-2′, H-6′), 6.90 (*d*, *J* = 8 Hz, H-3′, H-5′), 6.00 (*br s*, NH), 3.80 (*s*, OMe), 3.65 (*br t*, J = 7 Hz, 2 H-8′), 2.80 (*t*, J = 7 Hz, 2H-7′), 2.26 (*s*, 2 OAc).

N-*Acetyldiacetate* (**2h**). Mp 106–108° (Me_2CO–hexane). IR ν_{max}^{KBr} cm^{-1}: 1775, 1785, 1715, 1665. ^1H NMR (60 MHz, $CDCl_3$): δ 7.65–7.00 (*m*, H-3, H-4, H-5), 7.05 (*d*, *J* = 8 Hz, H-2′, H-6′), 6.78 (*d*, *J* = 8 Hz, H-3′, H-5′), 3.82 (*br t*, *J* = 7 Hz, 2H-8′), 3.70 (*s*, OMe), 2.80 (*t*, *J* = 7 Hz, 2H-7′), 2.30 (*s*, OAc), 2.20 (*s*, 2 OAc).

N-[8′-(4′-*Methoxyphenylethyl*)]-2-*hydroxy*-6-*methoxybenzoyl amide* (**2f**). Mp 55–56° (petrol). UV λ_{max}^{MeOH} nm: 220, 255, 312 (ε 18050, 7650, 2850). IR ν_{max}^{KBr} cm^{-1}: 3380, 1638. ^1H NMR (60 MHz, $CDCl_3$): δ 15.65 (*s*, OH), 8.35 (*br s*, NH), 7.20 (*m*, H-4), 7.18 (*d*, *J* = 8 Hz, H-2′, H-6′), 6.85 (*d*, *J* = 8 Hz, H-3′, H-5′), 6.60 (*dd*, *J* = 2, 7 Hz, H-3), 6.28 (*dd*, *J* = 2, 7 Hz, H-5), 3.70 (*s*, OMe), 3.68 (*br t*, *J* = 7 Hz, 2H-8′), 3.65 (*s*, OMe), 2.80 (*t*, *J* = 7 Hz, 2H-7′). ^{13}C NMR (20 MHz, $CDCl_3$): δ 170.0 (*s*, C-7), 164.5 (*s*, C-6), 158.7 (*s*, C-5), 158.5 (*s*, C-4′), 133.1 (*d*, C-4), 131.0 (*s*, C-1′), 129.8 (*d*, C-2′, C-6′), 114.2 (*d*, C-3′, C-5′), 111.7 (*d*, C-3), 104.1 (*s*, C-1), 101.0 (*d*, C-5), 56.0 (*q*, OMe), 55.3 (*q*, OMe), 40.5 (*t*, C-8′), 34.5 (*t*, C-7′). *MS m/z* (rel. int.): 301 (11), 151 (67), 134 (100), 121 (12).

(7S, 8S, 1′R, 5′R)-$\Delta^{8'}$-1′, 4′, 5′, 6′-*Tetrahydro*-3,4,5,5′-*tetramethoxy*-4′-*oxo*-7.O.2′,8.1′-*neolignan*(**4b**). For nomenclature and numbering of neolignans see ref. [15]. Mp 57–59° (MeOH). UV λ_{max}^{MeOH} nm: 258 (ε 10100). IV ν_{max}^{KBr} cm^{-1}: 1660. ^1H NMR (60 MHz, $CDCl_3$): δ 6.55 (*s*, H-2, H-6), 6.00–5.60 (*m*, H-8′), 5.56 (*s*, H-3′), 5.15 (*d*, *J* = 2 Hz, H-7), 5.27–4.90 (*m*, 2H-9′), 4.00–3.65 (*m*, H-5), 3.88 (*s*, 2 OMe), 3.85 (*s*, OMe), 3.60 (*s*, OMe), 2.85–2.00 (*m*, H-8, 2H-7′, 2H-6′), 1.20 (*d*, *J* = 7 Hz, 3H-9). ^{13}C NMR (20 MHz, $CDCl_3$): δ 196.6 (*s*, C-4′), 184.2 (*s*, C-2′), 153.6 (*s*, C-3, C-5), 136.2 (*d*, C-8′), 132.3 (*s*, C-4), 130.9 (*s*, C-1), 120.0 (*t*, C-9′), 101.4 (*d*, C-2, C-6), 101.0 (*d*, C-3′), 92.6 (*d*, C-7), 76.9 (*d*, C-5′), 60.9 (*q*, OMe), 59.0 (*q*, OMe), 56.4 (*q*, OMe), 55.3 (*q*, OMe), 48.8 (*s*, C-1′), 44.1 (*d*, C-8), 41.0 (*t*, C-7′), 31.8 (*t*, C-6′), 17.7 (*q*, C-9). MS *m/z* (rel. int.): $[M]^+$ 388 (69), 358 (21), 330 (42), 221 (100), 193 (22), 181 (41). CD (MeOH; *c* 0.01): $[\theta]_{317}^{max}$ + 13580, $[\theta]_{274}^{max}$ − 5240.

Antimicrobial assays. Assays with bacteria and yeasts were performed by the agar well diffusion method, using trypticase soy agar (Difco) and Sabouraud-dextrose agar (Difco) respectively. Qualitative evaluation of the antifungic activities was accomplished using a bioautographic assay [16]. Quantitative evaluation of the antifungic activities involved the utilization of a twofold serial dilution in Sabouraud-dextrose liquid medium. The conc. of samples ranged from 25 to 400 μg/ml. Solvent blanks were run against each test organism in all assays. Inhibition zone diameter in mm produced by amides (1 mg/ml DMSO) in antibacterial and antiyeast tests. *Candida albicans* **2e** 16 mm; *Saccharomyces cerevisiae* **2e** 10, **9** 8; *Bacillus cereus* **2e** 20, **2g** 15, **2h** 12, **9** 9; *Escherichia coli* **2e** 12, **2g** 11, **2h** 10, **9** 10; *Klebsiella pneumoniae* **2e** 11, **2g** 9, **2h** 8; *Salmonella gallinarum* **2e** 10; *Staphylococcus aureus* **2e** 25, **2g** 24, **2h** 20, **9** 8. Minimal inhibitory concentration of amides (μg/ml) in antifungic tests. *Cladosporium sphaerospermum* **2d** 200, **2e** 100, **2f** 400, **2g** 400, **2h** 400; *C. cladosporioides* **2d** 400, **2e** 50, **2f** 400, **2g** 100, **2h** 200; *C. cucummerinum* **2c** 400, **2d** 100, **2e** 50, **2f** 100, **2g** 50, **2h** 25.

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