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BIOACTIVE CHROMENES FROM *RHYNCHOLACIS PENICILLATA*

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Key Word Index—*Rhyncholacis penicillata*; podostemaceae; chromenes; rhynchonins A and B; structural elucidation; biological activity.

Abstract—Investigation of the aerial parts of *Rhyncholacis penicillata* afforded the new chromenes, 7-hydroxy-6-(3-methylbutyryl)-5-oxymethyl-chromene (rhynchonin A) and 7-hydroxy-6-(2-methylbutyryl)-6-oxymethylchromene (rhynchonin B). Structures were elucidated by spectroscopic methods and independent synthesis. Rhynchonin A showed broad insecticidal, acaricidal and nematicidal potency including strong biological activity against *Heliothis zea*.

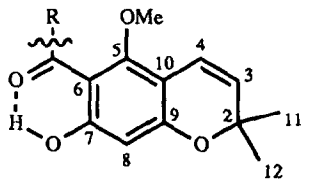
INTRODUCTION

Chromenes (benzopyrans) have been reported as constituents of a variety of higher plant species. The majority of these compounds have been isolated from the Asteraceae [1] but they also occur in the Rutaceae [2–5] and occasionally within the Cyperaceae [6] and Simaroubaceae [7]. We now report the isolation of two new chromenes (**1**, **2**) from the podostemaceae. The podostemaceae grow in fast-flowing tropical rivers, where they blossom and fruit during periods of low water levels [8, 9]. Until recently [10], no chemical data have been published on species of this family.

RESULTS AND DISCUSSION

TLC of an ether extract obtained from the aerial parts of *R. penicicllata* revealed the presence of triacylglycerols and phenolic compounds. The extract was subjected to column chromatography and subsequent HPLC on silica gel to yield two major phenolic compounds (**1** and **2**).

Compound **1** (M_r 290.1568, $C_{17}H_{22}O_4$) was obtained as a yellow, viscous oil, which slowly crystallized upon standing in the dark at 6°. The UV spectrum revealed a phenolic compound with an extended chromophore. ^{1}H NMR measurements revealed a proton vicinal to oxygenated positions of a phenolic unit (6.15, *s*, H-8), an oxymethyl group (3.75, *s*, 3H) and an array of signals characteristic of a 2,2-dimethyl-2*H*-chromene (Table 1), thus indicating a phloroglucinoid basic unit. Further structural elements were observed to be a 3-methylbutan-



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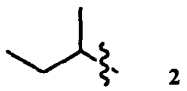

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Table 1. ^{1}H NMR spectral data of **1** and **2** (δ values, in $CDCl_3$, 400 MHz)

H	1	2
3	5.56 *d* (9.8)	5.57 *d* (10)
4	6.46 *d* (9.8)	6.47 *d* (10)
8	6.15 *s*	6.16 *s*
11	}1.41 *s*	}1.42 *s*
12		
2′	2.89 *d* (6.8)	3.61 *q* (6.7)
3′	2.23 *sept* (6.8)	1.77 *mp*; 1.41 *mp*
4′	0.93 *d* (6.8)	0.88 *t* (7.4)
5′		1.15 *d* (6.7)
O-Me	3.75 *s*	3.75 *s*
OH-7	13.33 *s*	13.22 *s*

Coupling constants (in Hz) in parentheses.

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oyl moiety (0.94, *d*, 6H; 2.23, *sept* 1H; 2.89, *d*, 2H) chelating a hydroxyl proton of the basic unit (13.33, *s*, 1H; IR *v* 1620 C=O). Finally, regional vicinity between the oxymethyl group and the allylic proton H-4 could be established by NOE, giving conclusive evidence of the proposed structure (**1**) for this compound.

Compound **2** (M_r 290.1568 $C_{17}H_{22}O_4$) was also obtained as a yellow, viscous oil. From spectroscopic evidence, it was found to be a structural isomer of **1**, bearing a 2-methyl-butanoyl moiety (3H, 0.88, *t*; 3H, 1.15, *d*; 1H, 1.41, *m*; 1H, 1.77, *m*; 1H, 3.61, *q*). All assignments were supported by ^{13}C NMR measurements (Table 2).

The 2,2-demethyl-2*H*-chromene structure assigned to **2** from its spectroscopic data was confirmed by an independent synthesis performed using the route depicted in Fig. 1. Methyl-2,6-dihydroxy-4-methoxy benzoate (**A**) was reacted with isovaleroyl chloride in nitrobenzene in the presence of $AlCl_3$ to product **B** of a Friedel–Crafts acylation which could be C-prenylated in the free aromatic position by means of 2-methyl-3-butene-2-ol and BF_3–etherate in dioxane to give **C**. When **C** was heated under reflux in 5% KOH, saponification of the ester function and decarboxylation occurred to yield the prenylated **D**. Finally **D** was transformed to the 2,2-dimethyl-2*H*-chromene **2** by oxidative cyclization with DDQ. The synthetic product was identical in all spectroscopic properties with the natural product.

In view of the novelty in finding chromenes with unusually long (methylbutyryl) side-chains as natural products, we named **1** and **2**, rhynchonins A and B, respectively. Rhynchonin A was tested for biological activity against *Heliotis zea*, an organism known to be sensitive to certain chromenoids [1]; it showed strong insecticidal activity at < 2 ppm [U. Kardorff, personal communication]. Moderate activity was also observed against other Lepidoptera, such as the cotton leafworm (*Prodenia litura*) and the diamond moth (*Plutella maculipennis*). Moderate activity was further observed on the housefly (*Musca domestica*) and the indifferent (not phytophagous) nematode species, *Caenorhabditis elegans*.

Table 2. ^{13}C NMR spectral data of **1**, **2*** and α-toxicarol†

C	1	2	α-toxicarol	
2	obscured	obscured	78.1	(C-6′)
3	116.4 *d*	116.5	} no value given	
4	128.1 *d*	128.1		
5	160.3 *s*	160.3	162.6	(C-7a)
6	107.2 *s*	107.3	101.5	(C-11a)
7	158.7 *s*	158.5	155.7	(C-11)
8	101.2 *d*	101.3	97.5	(C-10)
9	165.7 *s*	165.7	164.3	(C-9)
10	109.3 *s*	109.3	101.0	(C-8)
11	}28.3 *q*	}28.2	28.2	{(C-7′)
12				(C-8′)
1′	205.6 *s*	210.4		
2′	51.5 *t*	45.4		
3′	25.3 *d*	27.3		
4′	}22.7 *q*	11.9		
5′		17.0		
O-Me	63.1 *q*	63.5		

*Off-resonance-decoupled spectra were recorded in $CDCl_3$ at 100 MHz. Signal multiplicities ( ) were assigned utilizing the DEPT pulse sequence.

†Data refer to experiments utilizing noise-decoupling and single frequency off-resonance decoupling at 25.15 MHz [11].

EXPERIMENTAL

Mps: uncorr. IR spectra were recorded in $CHCl_3$ and as KBr disks, UV spectra in EtOH. NMR expts were

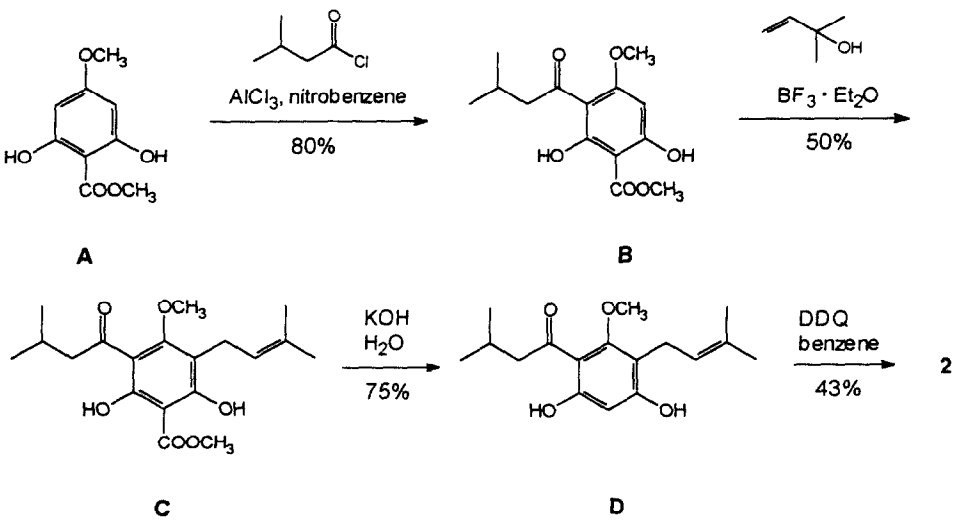

Fig. 1. Synthetic route for rhynchonin B.

performed at 400 and 100 MHz, measuring in $CDCl_3$. Silica gel used for prefractionation was Merck Kieselgel 60 (70–230 mesh). HPLC was carried out on a 250 × 8 mm Lichrospher Si (5 μm) column eluting at 8 ml min^{-1} using a RI detector.

Plant material. R. penicillata was collected in the vicinity of the Rio Caroni (Venezuela) in 1973 and identified by Dr M. Grubert, Universität Mainz, Germany. A herbarium specimen is deposited at the Institut für Spezielle Botanik der Universität Mainz (Dr Hecker; see Fig. 2).

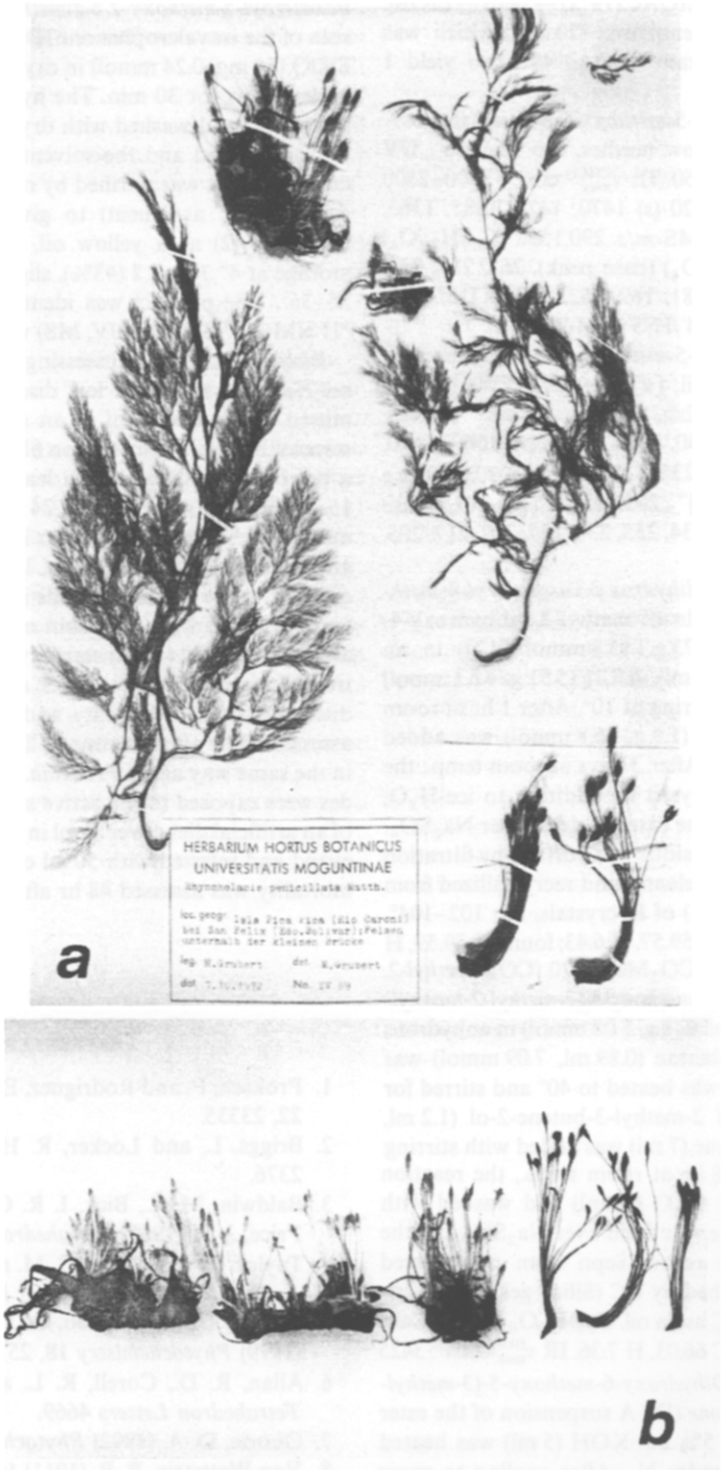

Fig. 2. *Rhyncholacis penicillata* Matth. (a) The submerged leaves are repeatedly pinnate, with numerous filform ultimate divisions. (b) Flowering and fruiting plants detached from the rocky substratum; out of the running water, the leaves soon wither and the short fleshy stem finally dessicates to the remnants at the base of the fruiting fascicles.

Extraction. Dry plant material (25 g) was milled and extracted with CH_2Cl_2 in a Soxhlet apparatus (4 hr). The extract (1.58 g) was analysed by TLC (silica gel; petrol–EtOAc (95:5); spray reagents: anisaldehyde–H_2SO_4 and Fast Blue B to show a triacylglycerol zone (R_f 0.2) and a major phenolic zone (R_f 0.3). CC using gradient elution (petrol–EtOAc (19:1) → petrol–EtOAc 9:1) yielding a crude phenol mix (70 mg), which was purified by HPLC (*n*-hexane–EtOAc 49:1) to yield **1** (25 mg) and **2** (12 mg).

7-Hydroxy-2,2-dimethyl-5-methoxy-6-3'-methylbutanoylchromene (**1**). Light yellow needles, mp 34.5–36°. UV λ_{max}^{EtOH} nm: 231sh, 264sh, 350. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300–2500 (*br*), 3050, 2950, 2870, 1620 (*s*) 1470, 1420, 1385, 1365, 1290, 1150, 1110, 1080. MS *m/z* 290.1568 ($C_{17}H_{22}O_4$) $[M]^+$, 275.1355 ($C_{16}H_{19}O_4$) (base peak), 268, 257, 243, 242, 233, 231, 203, 193, 181, 169, 162. NOE [Diff.; (%) O – Me → H-4 (1.52), H-4'/H-5' → H-2' (1.74).

7-Hydroxy-2,2-dimethyl-5-methoxy-6,2'-methylbutanoylchromene (**2**). Viscous oil, $[\alpha]_D^{20}$ – 2.8° ($CHCl_3$). UV λ_{max}^{EtOH} nm: 231sh, [264, 292sh, 351. IR ν_{max}^{film} cm^{-1}: 3300–2500 (*br*), 3070, 2980, 2940, 2880, 1625 (*s*), 1560, 1470, 1425), 1390, 1330, 1270, 1235, 1170, 1110, 1090. MS *m/z* 290.1568 ($C_{17}H_{22}O_4$) $[M]^+$, 275.1355 ($C_{16}H_{19}O_4$) (base peak), 268, 257, 243, 242, 234, 233, 231, 227, 219, 217, 205, 203, 193, 181, 169, 162.

Synthesis. Methyl-2,6-dihydroxy-3-isovaleryl-4-methoxybenzoate (**B**). To a soln of methyl-2,6-dihydroxy-4-methoxybenzoate (**A**) (2.73 g, 13.8 mmol [12]) in an hydrous nitrobenzene (25 ml), $AlCl_3$ (5.51 g, 41.3 mmol) was added slowly with stirring at 10°. After 1 hr at room temp., isovaleryl chloride (1.9 g, 15.8 mmol) was added slowly with stirring at 0°. After 3 days at room temp., the reaction mixt. was hydrolysed by addition to ice-H_2O, extracted with Et_2O and the extracts dried over Na_2SO_4. On evapn of solvent, the residue was purified by filtration over silica gel (CH_2Cl_2 as eluent) and recrystallized from EtOH to give 3.13 g (80%) of **B**, crystals, mp 102–104°. $C_{14}H_{18}O_6$ (283.3): Calc. C 59.57, H 6.43; found C 59.59, H 6.64. IR ν_{max}^{KBr} cm^{-1}: 1655 (CO_2Me), 1620 (CO). *Methyl-2,6-dihydroxy-3-isovaleryl-4-methoxy-5-(3-methyl-2-butenyl-benzoate* (**C**). To a soln of **B** (2 g, 7.08 mmol) in anhydrous dioxane (20 ml) BF_3-etherate (0.89 ml, 7.09 mmol) was added slowly. The soln was heated to 40° and stirred for 30 min, then a soln of 2-methyl-3-butene-2-ol (1.2 ml, 11.4 mmol) in dry dioxane (7 ml) was added with stirring at room temp. After 48 hr at room temp., the reaction mixt. was diluted with Et_2O (80 ml) and washed with H_2O. The organic phase was dried over Na_2SO_4 and the solvent distilled off *in vacuo*. Sepn from non-reacted product was accomplished by CC (Silica gel, CH_2Cl_2 as eluent) yielding 0.5 g of **C** as an oil. $C_{19}H_{26}O_6$ (350.4): Calc. C 65.13, H 7.48; found C 66.03, H 7.36. IR ν_{max}^{film} cm^{-1}: 3425 (OH), 1655 (CO). *2,4-Dihydroxy-6-methoxy-5-(3-methyl-2-butenyl)isovalerophenone* (**D**). A suspension of the ester (**C**) (0.35 g, 1 mmol) in 5% aq. KOH (5 ml) was heated under reflux for 4 hr under N_2. After cooling to room temp., the soln was acidified by addition of 5 M HCl and extracted several times with Et_2O. The combined extracts were washed with aq. $NaHCO_3$ soln and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product purified by CC (silica gel, Et_2O–petrol (40–60°), 1:1) to give 0.22 g (75%) of (**D**), crystals, mp 80–82°. $C_{17}H_{24}O_4$ (292.4): Calc. C 69.84, H 8.27; found C 70.69, H 8.17. IR ν_{max}^{KBr} cm^{-1}: 3305 (OH), 1630 (CO). *7-Hydroxy-6-isovaleroyl-5-methoxy-2,2-dimethyl-2H-chromene* (**2**). A soln of the isovalerophenone (**D**) (70 mg, 0.24 mmol) and DDQ (55 mg, 0.24 mmol) in dry C_6H_6 (8 ml) was heated under reflux for 30 min. The hydroquinone formed was filtered off and washed with dry C_6H_6. The C_6H_6 solns were combined and the solvent removed *in vacuo*. The crude product was purified by rapid filtration over silica gel (CH_2Cl_2 as eluent) to give the 2,2-dimethyl-2*H*-chromene (**2**) as a yellow oil, which crystallized after storage at 4° 30 mg **2** (43%), slightly yellow crystals, mp 35–36°. The product was identified in all respects (IR, ^1H NMR, ^{13}CNMR, UV, MS) with the natural product.

Biological tests. For assessing the ovolarvicidal effects on *Heliothis zea*, bean leaf discs (ϕ 22 mm) were submitted to dip-treatment in an aq. soln of the test substances. After arrangement on filter paper and transfer to a petri dish (ϕ 10 cm) each leaf disc was infested with 15–20 *Heliothis* eggs (max. 24 hr old). Egg hatch and mortality of hatching 1st instar larvae was assessed 96 hr after treatment. For *Prodenia*, larvae of 4th instar were exposed to the test compounds in petri dishes previously treated with an Me_2CO soln and the activity assessed after 4 hr. *Plutella* 4th instar larvae were exposed to dip-treated young cabbage leaves arranged in closed petri dishes (ϕ 4 cm). Mortality and feeding inhibition was assessed 48 hr after treatment. The *Musca* test was done in the same way as for *Prodenia*. *Caenorhabditis* nematodes were exposed to the active substances on the surface of an artificial diet cover (2 ml in Me_2CO soln, after being evapd and infested with 50 ml of nematode suspension); mortality was assessed 48 hr after treatment.

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