HIV-Inhibitory Prenylated Xanthones and Flavones from *Maclura tinctoria*[1]

Amiram Groweiss,[†] John H. Cardellina, II,[‡] and Michael R. Boyd*

Laboratory of Drug Discovery Research and Development, Developmental Therapeutic Program, Division of Cancer Treatment and Diagnosis, Frederick Cancer Research and Development Center, National Cancer Institute, Frederick, Maryland 21702-1201

Received April 14, 2000

The organic extract of the plant *Maclura tinctoria* exhibited moderate anti-HIV activity. Seven prenylated phenolic derivatives were isolated from the active fractions and characterized by spectroanalytical methods. New compounds macluraxanthone B (**1**), macluraxanthone C (**2**), and dihydrocudraflavone B (**8**) were identified.

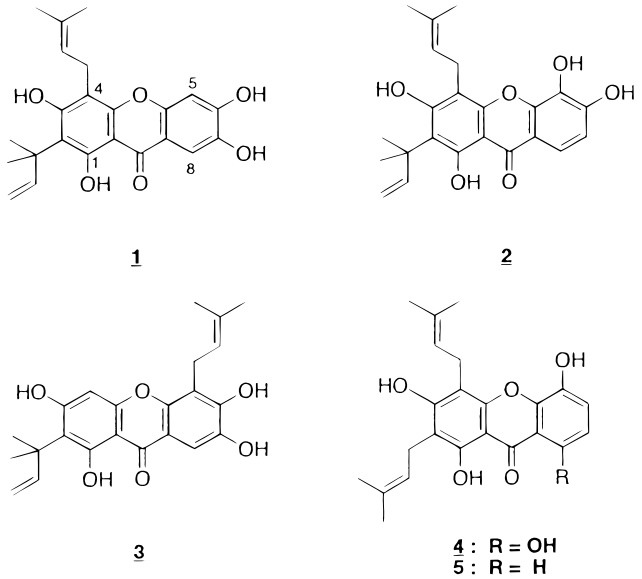
The search for natural products with HIV-inhibitory activity has been an ongoing project in our laboratory for the past decade. This effort has previously led to the isolation of several drug development candidates, such as the antiviral compounds michellamine B from *Ancistrocladus korupensis*,[2] calanolides A and B from *Calophyllum lanigerum*,[3] and cyanovirin-N from the cyanobacterium *Nostoc ellipsosporum*.[4] Among the HIV-inhibitory leads emanating from the NCI primary anti-HIV screen[5] was the organic extract of the bark from *Maclura tinctoria* (L.) Steud. (Moraceae), which showed moderate in vitro antiviral activity and was, therefore, selected for bioassay-guided fractionation.

Previous work on another species of the genus *Maclura*, namely *M. pomifera* (known as "Osage Orange") resulted in the isolation of several prenylated xanthones, such as macluraxanthone[6] and alvaxanthone.[7] In this report, we describe the isolation and structure determination of seven prenylated xanthones and flavones, including macluraxanthone B (**1**), macluraxanthone C (**2**), and dihydrocudraflavone B (**8**), which are new natural products.

Bioassay-guided fractionation of the organic extract (CH_2Cl_2−CH_3OH, 1:1) tracked the HIV-inhibitory activity to two fractions from a solvent/solvent partition scheme (see Experimental Section). Further separation of the active fractions by size exclusion (Sephadex LH-20) and vacuum flash chromatography and final purification by HPLC afforded seven compounds (**1**, **2**, **4**−**8**), all obtained as yellow solids.

The molecular formula of compound **1**, which we have named macluraxanthone B, was established by HREIMS as $C_{23}H_{24}O_6$. The UV, IR, ^{1}H NMR, and ^{13}C NMR spectra showed signals that were attributed to a carbonyl, eight carbon−carbon double bonds, and four exchangeable protons. Comparison with known compounds[8] indicated that the structure of **1** was composed of a xanthone ring system, containing four hydroxyl and two prenyl substituents. The two C_5 groups were identified by NMR as an isopent-2-enyl (methyls at δ_H 1.63 and 1.84, a vinylic proton at δ_H 5.10) and 1,1′-dimethylallyl (a two-methyl signal at δ_H 1.57, three protons in an ABX system at δ_H 4.90, 4.95, and 6.35). The remaining two positions on the xanthone ring were occupied by two aromatic protons (δ_H 7.37 and 6.86, both singlets). The positioning of the substituents on the ring system was based on chemical shift considerations and, mainly, on the results of HMBC experiments in $CDCl_3$, CD_3OD, and DMSO-d_6. Especially informative were the data acquired in DMSO-d_6, which allowed spectral assignments to start from the very characteristic signal at δ_H 14.2 (1-OH), which showed correlations with C-1 (δ 159.8), C-9a (δ 102.3), and C-2 (δ 115.3). These assignments further established that the 1,1′-dimethylallyl group was located at C-2, the isopent-2-enyl at C-4, and another OH at C-3. Therefore, the remaining two hydroxyls were located on another ring. Lack of either ortho or meta coupling between the two aromatic protons suggested that they could only be positioned at C-5 and C-8. The 1,4-ratio between these protons was indeed proven by a long-range COSY experiment, thereby completing the structure determination of **1** as shown in Scheme 1. A literature survey revealed several geometrical isomers of **1**, such as alvaxanthone,[7] cudraxanthone L (**3**) from *Cudrania tricuspidata*,[9] and gerontoxanthone I.[10] However, macluraxanthone B is a new natural product.

Scheme 1



Macluraxanthone C (**2**), another regioisomer, was obtained in another fraction as a mixture with **1** and exhibited very similar spectral properties. Subtraction of the NMR peaks of **1** from the mixture showed that the only difference between the structures was a 5,6-disposition of hydroxyl groups, instead of the 6,7-dihydroxyl array of **1**.

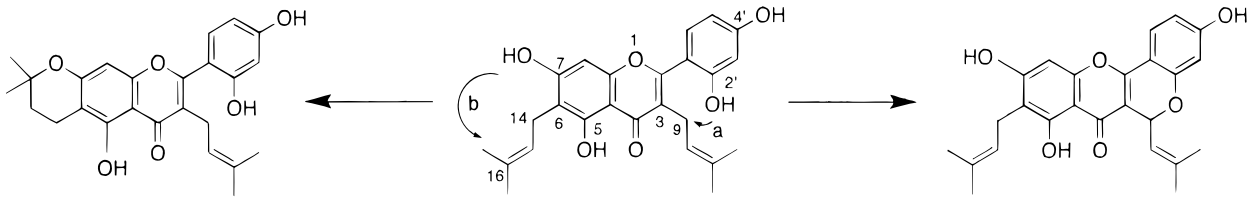
* To whom correspondence should be addressed. Tel.: (301)846-5393. Fax: (301)846-6177. E-mail: boyd@dtpax2.ncifcrf.gov.

[†] On sabbatical leave from TAMI (IMI) Institute for Research and Development, Haifa Bay 26111, Israel.

[‡] Present address: Council of Responsible Nutrition, 1875 Eye Street NW, Suite 400, Washington D.C. 20006-5409.

10.1021/np000175m
Published on Web 09/07/2000

Scheme 2



8 **6** **7**

Table 1. Anti-HIV Testing Results of Compounds **1**, **2**, and **4**−**8**

compound	HIV-inhibitory activity EC_{50} (μg/mL)	toxicity IC_{50} (μg/mL)
1	1−2	2.2−3.3
2	1.3−2.2	3.7
4	I[a]	5.3
5	I[a]	4.9
6	I[a]	5
7	7.5	17
8	I[a]	12

[a] Inactive.

An ortho coupling constant was measured between two protons at δ_H 6.92 and 7.69, and the positioning of those aromatic protons at H-7 and H-8, respectively, was supported by an HMBC correlation between the signal at δ_H 7.69 (H-8) and the carbonyl signal at δ_C 180.9 (C-9). The spectral assignments of **2** were based on comparison with the reported NMR data of gerontoxanthone I.[10] We also isolated two other known bis-prenylated xanthones, gartanin (**4**) and 8-desoxygartanin (**5**).[11]

Isoprenylated flavones are also very common in the family Moraceae.[7,12] In our investigation we have isolated three such compounds (Scheme 2). NMR and spectral comparisons showed that two of those were reported earlier; cudraflavone C (**6**) was a constituent of *C. tricuspidata*,[13] while isocyclomulberrin (**7**) was isolated from *Artocarpus altilis*.[14] The third compound (**8**), $C_{25}H_{26}O_6$, was found to be a new natural product. 2D NMR experiments showed similarities to **6** in many aspects; however, instead of the two C_5 units, **8** contained only one such unit. The other C_5 unit comprised a 2,2′-dimethylchromane ring system with the 7-OH, as indicated by the chemical shifts of the geminal methyls (δ_H 1.31) and the oxygen-bearing carbon C-16 (δ_C 76.4). A closely related compound, cudraflavone B,[15,16] was found to differ from **8** by a Δ^{14} double bond, while compound **8** had two methylenes at C-14 and C-15 (triplets at δ_H 2.62 and 1.81, δ_C 16.1 and 31.3, respectively). It is interesting to note that **6** could be the precursor of both **7** and **8**; a ring closure between 2′-OH and the allylic C-9 forms compound **7** (Scheme 2, pathway a), while pathway b illustrates the formation of compound **8**.

The seven compounds were tested in the primary anti-HIV screen and three compounds exhibited consistent activity (see Table 1). Macluraxanthones B (**1**) and C (**2**) showed the best potential, with EC_{50} levels of 1−2.2 μg/mL; isocyclomulberrin (**7**) had an EC_{50} level of 7.5 μg/mL, although dihydrocudraflavone B (**8**) showed only marginal activity. The catechol functionality of **1** and **2** would appear to proffer enhanced HIV-inhibitory activity. However, much like many other prenylated phenolic compounds discovered in our laboratory, such as the guttiferones[17−19] or vismiaphenone D,[20] all seven compounds from *M. tinctoria* exhibited very high toxicity toward the CEM-SS host cells, with IC_{50} levels of 2.2−17 μg/mL.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian VXR-500 spectrometer using $CDCl_3$, DMSO-d_6, and CD_3OD as solvents and internal standards. Infrared spectra were measured on a Perkin-Elmer 267 spectrometer, and ultraviolet spectra were obtained with a Beckman 34 spectrophotometer. Mass spectra were recorded on VG Micromass ZAB 2F and Finnigan Mat 95 mass spectrometers.

Plant Material. The bark of *M. tinctoria* (L.) Steud was collected by D. C. Daly, under contract to the National Cancer Institute, near Loreto, Prov. Maynas (longitude 73° 45.0″ W, latitude 4° 55.02″ S), Peru, in February 1988. A voucher specimen (Q65-T405) is maintained at the New York Botanical Garden. Extraction of 179 g dried, ground plant material with CH_2Cl_2−MeOH (1:1) and MeOH yielded 11.12 g crude extract.

Fractionation of the Crude Extract. The crude organic extract (8.46 g) was partitioned according to the following protocol: partition between 90% aqueous MeOH and hexane (affording 2.97 g), 80% aqueous MeOH and CCl_4 (0.75 g), and 70% MeOH/H_2O and $CHCl_3$ (0.91 g). Then the MeOH was removed in vacuo, and the aqueous residue was extracted with EtOAc to give 0.78 g of organic soluble material. Finally, the H_2O phase was freeze-dried to give a residue of 3.01 g. The antiviral activity was tracked to the CCl_4 (EC_{50} 10 μg/mL) and the $CHCl_3$ (EC_{50} 7 μg/mL) fractions.

Separation of the CCl_4 Fraction and Isolation of Compounds 1, 2, 4, 5, and 8. The CCl_4 fraction (748 mg) was first subjected to gel permeation through a Sephadex LH-20 column (elution with CH_2Cl_2−MeOH−hexane, 5:1:2); 30 fractions were collected. The two most active ones (55 mg, EC_{50} 2.5 μg/mL) were combined and further separated by flash chromatography over a short column containing bulk cyano-bonded adsorbent. Elution with mixtures of hexane−*i*PrOH led to the isolation of two active xanthones, **1** (7 mg) in pure form, and **2** (20 mg), as a mixture with **1**, respectively. A cytotoxic fraction from the Sephadex LH-20 separation (92 mg, IC_{50} 12 μg/mL) was separated by two subsequent chromatographies over Si gel and bulk C_{18}-bonded phase and HPLC (Rainin Dynamax-NH_2 column, elution with $CHCl_3$−MeOH, 17:3) to give two other prenylated xanthones, gartanin (**4**, 8.4 mg) and desoxygartanin (**5**, 5.5 mg). Finally, another anti-HIV active fraction from the initial Sephadex LH-20 column (16 mg, EC_{50} 12 μg/mL) was separated by flash cyano-bonded-phase liquid chromatography, followed by HPLC separation (Rainin Dynamax-CN column, elution with hexane−*i*PrOH, 3:1). Pure dihydrocudraflavone B (**8**, 7 mg) was obtained.

Separation of the $CHCl_3$ Fraction and Isolation of Compounds 6−**8.** Gel permeation chromatography of the $CHCl_3$ fraction (913 mg) through a Sephadex LH-20 column (elution with MeOH) gave two fractions with significant anti-HIV activity. The first fraction (168 mg, EC_{50} 4−5 μg/mL) was subjected to a separation scheme that included another Sephadex LH-20 chromatography (elution with $CHCl_3$/MeOH, 1:1), followed by flash chromatography over a cyano-bonded phase column (elution with mixtures of hexane−*i*PrOH) and HPLC purification (on a 1 × 25 cm Rainin Dynamax-CN

column; elution with hexane−*i*PrOH, 3:1). This led to the isolation of two known compounds, cudraflavone C (**6**, 15 mg) and isocyclomulberrin (**7**, 9 mg) in pure form. The second active fraction from the initial Sephadex LH-20 chromatography of the $CHCl_3$-soluble portion (257 mg, EC_{50} 3 μg/mL), was subjected to the same separation methodology, which led to the isolation of more dihydrocudraflavone B (**8**, 10 mg).

Macluraxanthone B (1): yellow amorphous solid, UV (MeOH), λ_{max} nm (log ϵ) 376 (3.99), 320 (4.16), 259 (4.38), 236 (4.36), 206 (4.44); IR (CH_2Cl_2) ν_{max} 3390, 2929, 1634, 1587, 1480, 1294, 1200, 1168, 1074, 935, 847 cm^{-1}; HREIMS *m/z* 396.1525 (M^+, calcd for $C_{23}H_{24}O_6$: 396.1572); EIMS *m/z* (relative abundance) 396 (M^+,100), 381 (89), 353 (15), 341 (39), 328 (32), 313 (42), 285 (24), 273 (30); ^{13}C NMR (DMSO-d_6) δ 180.0 (C-9), 160.8 (C-3), 159.8 (C-1), 154.4 (C-7), 152.9 (C-4a), 150.9 (C-4b), 150.7 (C-14), 144.0 (C-6), 131.0 (C-18), 122.6 (C-17), 115.3 (C-2), 111.8 (C-8a), 108.7 (C-15), 108.4 (C-8), 106.6 (C-4), 102.7 (C-5), 102.3 (C-9a), 41.1 (C-11), 29.0 (C-12 & C-13), 25.8 (C-20), 21.8 (C-16), 18.1 (C-19); ^1H NMR (DMSO-d_6) δ 14.2 s, 10.70 br s, 9.70 br s, 9.10 s (4 D_2O exchangeable protons, 1-OH, 6-OH, 7-OH & 3-OH, respectively), 7.37 s (H-8), 6.86 s (H-5), 6.35 (dd, J = 17.9, 10.7 Hz, H-14), 5.10 (br t, 7.1, 1.4, H-17), 4.95 (dd, 17.9, <1, H-15), 4.90 (dd, 10.5, <1, H-15′), 3.45 (d, 7.1, 2H, H-16,16′), 1.84 (br s, 3H, Me-19), 1.63 (br s, 3H, Me-20) and 1.57 (br s, 6H, Me-12, -13).

Macluraxanthone C (2): yellow amorphous solid; HREIMS *m/z* 396.1589 (M^+, calcd for $C_{23}H_{24}O_6$: 396.1572); EIMS *m/z* (relative abundance) 396 (M^+, 71), 381 (100), 341 (31), 325 (35), 313 (30), 285 (20); ^{13}C NMR ($CDCl_3$) δ 180.9 (C-9), 164.0 (C-3), 160.6 (C-1), 154.6 (C-4a), 149.7 (C-14), 149.2 (C-6), 145.0 (C-4b), 131.8 (C-18), 130.3 (C-5), 122.9 (C-17), 118.0 (C-8), 114.1 (C-8a), 113.6 (C-15), 112.4 (C-7), 106.7 (C-2), 103.7 (C-9a), 102.1 (C-4), 43.8 (C-11), 27.0 (C-12 & C-13), 25.6 (C-20), 22.4 (C-16), 17.8 (C-19); ^1H NMR ($CDCl_3$) δ 13.0 s, 7.65 s (D_2O exchangeable protons, 1-OH and 3-OH, respectively), 7.69 (d, J = 8.6 Hz , H-8), 6.92 (d, 8.6, H-7), 6.48 (dd, 17.7, 10.5, H-14), 5.49 (d, 17.7, H-15), 5.38 (dd, 10.5, <1, H-15′), 5.27 (br t, 7, H-17), 3.42 (d, 6.8, 2H, H-16,16′), 1.81 (br s, 3H, Me-19), 1.70 (br s, 3H, Me-20) and 1.63 (br s, 6H, Me-12, -13).

Dihydrocudraflavone B (8): viscous yellow solid; UV (MeOH), λ_{max} nm (log ϵ) 306 (3.78), 262 (4.41), 233 (4.03), 206 (4.36); IR (CH_2Cl_2) ν_{max} 3567, 1651, 1618, 1457, 1375, 1217, 1158, 1094 cm^{-1}; HREIMS *m/z* 422.1733 (M^+, calcd for $C_{25}H_{26}O_6$: 422.1729); EIMS *m/z* (relative abundance) 422 (M^+, 30), 405 (18), 379 (70), 323 (20), 293 (21), 167 (16), 149 (100), 71 (38), 69 (37), 57 (44), 55 (38); ^{13}C NMR (DMSO-d_6) δ 182.0 (C-4), 162.2 (C-2), 160.7 (C-2′), 160.0 (C-7), 158.6 (C-5), 156.7 (C-4′), 155.6 (C-8a), 131.4 (C-11 & C-6′), 121.9 (C-10), 119.9 (C-3), 111.4 (C-1′), 107.0 (C-5′), 104.5 (C-6), 103.5 (C-4a), 102.9 (C-3′), 94.4 (C-8), 76.4 (C-16), 31.3 (C-15), 26.6 (C-17 & C-18), 25.7 (C-13), 23.9 (C-9), 17.6 (C-12), 16.1 (C-14); ^1H NMR (DMSO-d_6) δ 13.42 s, 9.86 s, 9.77 s (3 D_2O exchangeable protons, H-5, H-2′, and H-4′, respectively), 7.06 (d, J = 8.3 Hz, H-6′), 6.42 (d, 2.2, H-3′), 6.33 (dd, 8.3, 2.2, H-5′), 6.29 s (H-8), 5.01 (dt, 7.0, 1.3, H-10), 2.98 (d, 7, 2H, H-9, -9′), 2.62 (t, 6.8, 2H, H-14, -14′), 1.81 (t, 6.8, 2H, H-15, -15′),1.54 (br s, 3H, Me-13), 1.36 (br s, 3H, Me-12), 1.31 (s, 6H, Me-17, -18).

Acknowledgment. We thank the New York Botanical Garden and G. M. Cragg for the plant collection, T. McCloud for extraction, J. B. McMahon and R. J. Gulakowski for the anti-HIV assays, and Glen Gray for the mass spectrometry data.

Supporting Information Available: Full spectroanalytical details, including NMR data (COSY, HMQC, and HMBC correlations) and full NMR assignments of signals of compounds **1**−**2** and **4**−**8** (Tables 2−8). This material is available free of charge via the Internet at http://pubs.acs.org.

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NP000175M