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ISOLATION OF A NOVEL CYTOTOXIC POLYACETYLENE FROM A TRADITIONAL ANTHELMINTIC MEDICINAL PLANT, *MINQUARTIA GUIANENSIS*

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ABSTRACT.—The stem bark of *Minquartia guianensis* is used by the Quijos Quichua people of Ecuador's Amazonian lowlands in an infusion drunk to treat intestinal parasitic infections, lung cancer, and tuberculosis and is applied topically to treat muscular pain and skin irritations. Using the in vitro P-388 murine lymphocytic leukemia cytotoxicity bioassay to guide fractionation of the $CHCl_3$ extract of *M. guianensis* stem bark, the novel cytotoxic polyacetylene (−)-17-hydroxy-9,11,13,15-octadecatetraynoic acid [**1**] was isolated. The P-388 ED_{50} of the pure compound is 0.18 μg/ml, and it is also active in the brine shrimp larvicidal bioassay, with an LC_{50} of 5.06 μg/ml (95% confidence interval 3.68–6.98). These biological activities could account for the alleged efficacy of the plant in folk usage.

Ethnopharmacological field work was conducted for ten weeks with the Quijos Quichua of Ecuador's Amazonian lowlands. Traditional healers living in the upper regions of the Napo, Arajuno, and Huambuno rivers (1°S, 77°30′W, 450 m altitude) were interviewed, and voucher specimens of each of the 120 species of medicinal plants that they described were collected.

Bulk collections (5–10 kg dry wt) were made of several plants that were being used to treat symptoms suggestive of parasitic infections. Studies on the prevalence of helminthiasis in the humid tropical rural areas of the Americas have found that at least 90% of the population harbors at least one parasitic species, and in some areas at least 60% of the population harbors two or more species (1,2). Protozoal, fungal, bacterial, and viral infections are also widespread and have a severe impact on the health of the rural population. Parasitic infections present symptoms which the traditional healers can readily recognize, prescribe for, and observe for the results of their treatment. This facilitates the correlation between chemical constituents, demonstrated pharmacological activities, and traditional uses, increasing the chances of discovering effective medicines through the investigation of ethnomedical observations.

One of the most potent traditional anthelmintics of the Quijos Quichua is an infusion of the stem bark of *Minquartia guianensis* Aubl. (Olacaceae). This is an understory tree of the tropical lowlands of Central and South America. The Quijos Quichua soak approximately 500 g of the fibrous inner bark in approximately 1 liter of hot water to make a coffee-with-milk-colored infusion. The outer stem bark is considered too strong to be used. The usual dose is 1 liter for Quichua people or 0.5 liter for "colonials" (i.e., all non-natives) taken one time. This medicine is given only to adults. According to the traditional healer, in 2 to 3 h bowel movement should start; if not, sugar-water is given to the patient to make him vomit twice, after which the stool will pass. First, small parasites are eliminated, followed after 3 days by large worms. The stool will be black for 3 days. The inner bark is also pulverized, wrapped in a cloth, and applied topically as a poultice to treat sore limbs, sore kidneys, or skin irritations. It is said to cause the skin to turn black. In a follow-up visit to the same Quijos Quichua informant, it was

learned that *M. guianensis* inner bark decoction is also used to treat lung cancer and tuberculosis.[1]

Ethnobotanical literature reports the use of *M. guianensis* by the closely allied Canelos Quichua to treat stomach aches,[2] by the Napo Quichua as an anthelmintic, antipruritic, antiseptic, and antifungal (3) (recorded only under the Quichua common name with no botanical identification), by the Napo Quichua and Waorani as a fish poison (4), and in Brazil as a substitute for sandalwood oil in the treatment of affections of the urogenital organs (5). Throughout the tropics the wood is used for construction, as it is hard and extremely resistant to rot (6–8). The above uses suggest that *Minquartia* possesses rather non-specific effects.

Chemical literature on *Minquartia*, other than a report of a greenish or pale-blue essential oil of "cinnamon-like smell with a touch of that of rose oil" (5), appears to be lacking. In the family Olacaceae, triglycerides with C_{18} acetylenic fatty acids are known to occur in the roots, stems, and leaves of *Ximenia americana* and *Olax stricta* (9). The aromatic eudesmane-type sesquiterpene, manicol, which possesses weak antileukemic activity (10,11), and the sesquiterpene alkaloids, manicolines A and B (12), have been isolated from the root bark of *Dulacia guianensis*. The tropane alkaloid scopolamine has been isolated from the fruits of *Heisteria latifolia* (13). Triterpenoid saponins, prunasin-like cyanogenic glycosides, and condensed flavonoid tannins are also known from the family (6,14).

In order to determine the major toxic principle of *M. guianensis*, extracts of the stem bark were tested for invertebrate toxicity using the brine shrimp larvicidal bioassay (15) and for cytotoxicity using the in vitro P-388 murine lymphocytic leukemia bioassay (16). The most active extract was then subjected to bioassay-guided fractionation, employing the cytotoxicity bioassay. In the search for new cytotoxic, potentially antitumor, compounds from plants, it has been noted that selection on the basis of known prior anthelmintic use increases the chances of success threefold over random screening (17). This provided an additional reason for examining cytotoxicity in this study.

RESULTS AND DISCUSSION

An aqueous extract of the stem bark of *M. guianensis* gave a brine shrimp bioassay LC_{50} of 25.15 μg/ml and a P-388 ED_{50} of 3.19 μg/ml, while the $CHCl_3$ extract gave a brine shrimp bioassay LC_{50} of 20.4 μg/ml and a P-388 ED_{50} of 2.16 μg/ml. Guided by the P-388 bioassay, the $CHCl_3$ extract of 1.0 kg of the stem bark of *M. guianensis* was fractionated by cc and vacuum liquid chromatography over Si gel with gradient elution to afford 7.0 g of crystalline material estimated by tlc to be at least 80% pure (a yield of approximately 0.6%). Recrystallization of 100 mg of crude crystals from CH_2Cl_2 afforded pale gray-yellow needle-like crystals of (−)-17-hydroxy-9,11,13,15-octadecatetraynoic acid [**1**], a novel naturally occurring polyacetylene to which we have given the trivial name minquartynoic acid.

Minquartynoic acid has a melting point of 95°. The uv spectrum shows an intense absorbance characteristic of conjugated alkynes with a λ max in MeOH of 239 nm (log ε 5.46). The ir spectrum indicates the presence of hydroxyl (ν max 3350 cm^{-1}), carboxylic acid (broad O-H stretch at ν max 3300–2500 cm^{-1} and C=O stretch at ν max 1709 cm^{-1}), and alkyne (ν max 2221 cm^{-1}) functionalities. The eims shows an $[M]^+$ at *m/z* 284 (20%), with a prominent $[M-H_2O]^+$ at *m/z* 266 (94%), and clusters of

[1]W. Wilbert and D. Neill, "Medical Ethnobotany of the Quijos-Quichua of the Upper Rio Napo, Amazonian Ecuador," unpublished manuscript, 1987.

[2]M.J. Shemluck, unpublished manuscript, no date.

peaks 14 amu apart characteristic of an aliphatic chain. The molecular ion was confirmed by low eV eims [*m/z* 284 (31%)], negative ion cims [*m/z* 284 (100%)], and fabms [*m/z* $[M+Li]^+$ 291 (82%)], $[M-H+2Li]^+$ 297 (84%)].

The ^{1}H-nmr spectrum (Table 1) shows some solvent effects useful to distinguish overlapping peaks. In CD_3OD the triplets of H-2 at δ 2.37 and H-8 at δ 2.28 are clearly resolved, but the signals of H-7 at δ 1.60 and H-3 at δ 1.54 overlap. In $CDCl_3$ the triplets (with some second-order splitting) of H-7 at δ 1.64 and H-3 at δ 1.55 are clearly resolved, while the triplets of H-2 at δ 2.36 and H-8 at δ 2.31 show some overlap. The ^{1}H-nmr spectrum was assigned according to a homonuclear COSY experiment, which clearly showed the coupling between the H-18 doublet at δ 1.40 and the H-17 quartet at δ 4.52, and allowed the assignment of the overlapping broad triplets of H-7 at δ 1.60 and H-3 at δ 1.54 through their coupling to the triplets of H-8 at δ 2.28 and H-2 at δ 2.37.

TABLE 1. ^{1}H-nmr Spectral Data for Minquartynoic Acid [**1**].[a]

Proton	$CDCl_3$	CD_3OD
2	2.36 t (7.4)	2.37 t (6.9)
3	1.55 br t (7.4)	1.54 br t (7.0)
4+5+6	1.30–1.43 m	1.30–1.38 m
7	1.64 br t (7.1)	1.60 br t (7.1)
8	2.31 t (6.9)	2.28 t (7.4)
17	4.59 q (6.7)	4.52 q (6.7)
18	1.49 d (6.8)	1.40 d (6.7)

[a]Obtained at 300 MHz, δ TMS = 0 ppm. Data are expressed as δ H, multiplicity (*J* in Hz).

The ^{13}C-nmr spectrum (Table 2) was assigned by chemical shift theory and by comparison with published chemical shift values for closely related hydroxylated and carboxylated polyacetylenes (18–22). Further evidence for the assignments was provided by an APT experiment which indicated that the signal at δ 24.02 represents a carbon with three attached protons (C-18), while the signal at δ 58.81 represents a carbon with one attached proton (C-17).

TABLE 2. ^{13}C-nmr Spectral Data for Minquartynoic Acid [**1**].[a]

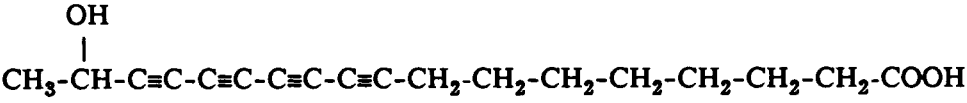
Carbon	δC	Carbon	δC
1	177.63	10	68.44[d]
2	34.91	11	64.16[e]
3	26.01	12	60.86[f]
4	30.02[b]	13	60.35[f]
5	29.88[b]	14	63.71[e]
6	29.78[b]	15	65.89[d]
7	28.97	16	81.61[c]
8	19.91	17	58.81
9	82.85[c]	18	24.02

[a]Obtained at 90.8 MHz in CD_3OD, δ TMS = 0 ppm.
[b,c,d,e,f]Assignments are interchangeable due to proximity of signals.

Minquartynoic acid has $[\alpha]^{25}D$ −29° (*c* = 0.1, MeOH) due to the chiral center at C-17. Optical activity was observed in the cd spectrum as well; however, it does not show a Cotton effect on the basis of which the absolute configuration could be deter-

mined. Oxidative degradation experiments were performed in an attempt to simplify the molecule, but no useful results were obtained. Work continues on the determination of the absolute configuration of **1**.

The P-388 ED_{50} of **1** is 0.18 μg/ml. In pure form it is unstable to light, air, and temperatures over 100°: the crystals change from gray to sapphire blue (ED_{50} 0.24 μg/ml after 1 h of uv irradiation), and a solution of pure **1** in $CHCl_3$, MeOH, or DMSO will change from colorless to red-brown (ED_{50} 0.53 μg/ml after 1 week of exposure to light and air). The brine shrimp bioassay LC_{50} of **1** is 5.06 μg/ml (95% confidence interval 3.68–6.98). The high levels of biological activities of minquartynoic acid (20 times the accepted P-388 minimum of 4μg/ml, and 100 times the accepted brine shrimp bioassay minimum of 1000 μg/ml) could account for the alleged efficacy in folk usage of *M. guianensis*. The high yield of 0.6% could prove to be economically significant.



1

Based on the biogenetic studies of Bohlmann *et al.* (23), it can be postulated that **1** is biosynthesized by the acetate pathway, with its fatty acid precursor oleic acid becoming unsaturated by successive dehydrogenation steps. The secondary hydroxyl moiety would probably result from epoxidation and reduction of a terminal double bond. The possession of four conjugated triple bonds and a hydroxyl group at C-17 instead of C-8 distinguish **1** from other polyacetylenes occurring in the Olacaceae and closely related Santalaceae (23–29). Tetra-acetylenic acids with a terminal methyl-secondary alcohol have been isolated from fungi (30) but do not have the seven-membered methylene chain which is characteristic of many of the polyacetylenes of higher plants. If more members of the Olacaceae are studied to determine the distribution of this chemical class, the type of polyacetylene represented by **1** could prove to be of chemotaxonomic significance.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Uv spectra were taken in MeOH and hexane on a Beckman DU-7 spectrophotometer. Ir spectra were taken as a KBr pellet on a Nicolet MX-1 FT-IR spectrophotometer. Low resolution mass spectra were recorded on a Varian MAT-112S mass spectrometer. Negative ion cims, using CH_4 as the reagent gas, was recorded on a Finnigan 4510 mass spectrometer. Fabms, using 3-nitrobenzoic acid/LiI in glycerol, was provided by the Midwest Center for Mass Spectrometry, University of Nebraska. ^{13}C-nmr spectra were recorded with a Nicolet NT-360 spectrometer operating at 90.8 MHz. ^{1}H-nmr and COSY homonuclear correlation spectra were recorded on a Varian XL-300 spectrometer operating at 300 MHz. TMS was used as an internal standard. Optical rotation was obtained with a Perkin-Elmer 241 polarimeter. Cd was measured with a Jasco J-40A spectropolarimeter.

Extracts were chromatographed on columns packed with Kieselgel 60, 70–230, or 270–400 mesh (Merck, Darmstadt, W. Germany). Tlc was performed on Si gel 60F-254 coated aluminum-backed sheets (Merck), and detection was performed at 254 and 366 nm after spraying with H_2SO_4 (50% in EtOH). All solvents were redistilled.

PLANT MATERIAL.—Stem bark of *M. guianensis* was collected in the Napo River valley of Ecuador in November 1985 and dried in partial shade. A voucher specimen was collected from the same tree at the same time and was identified by comparison with herbarium specimens. Voucher specimens are deposited in the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, with duplicates in the herbarium of the Missouri Botanical Garden, St. Louis, and the herbarium of the Dirección Nacional Forestal, Ministerio de Agricultura y Ganadería (National Forest Service, Ministry of Agriculture and Animal Husbandry), Conocoto, Ecuador.

BIOASSAYS.—The brine shrimp bioassay (15) for larvicidal activity was performed with the following minor modifications. The growth medium brine was neutralized with HCl to pH 7.0 and aerated to enhance hatching of the brine shrimp eggs. Test substances and colchicine as the positive control were dissolved in DMSO and diluted with double-distilled H_2O, such that following addition of the nauplii in 2.85 ml of brine, final test concentrations of 1000, 100, 10, and 1 μg/ml in 0.5% DMSO were obtained. The poor solubility of pure minquartynoic acid in brine necessitated the preparation of a coprecipitate with polyvinylpyrrolidone (PVP). A sample of minquartynoic acid (20 mg) was dissolved in MeOH, and four times that amount of PVP (80 mg) was dissolved in MeOH; then the solutions were mixed, dried under reduced pressure in a rotary evaporator, and resuspended/dissolved in double-distilled H_2O (2.0 ml). A control sample containing PVP (400 μg/ml) in H_2O did not cause significant brine shrimp mortality. Test concentrations and brine shrimp nauplii were incubated in 15-ml test tubes with light and air for 24 h, the number of dead brine shrimp in each tube was counted, and then EtOH (95%, 3 ml) was added to kill the remaining brine shrimp to facilitate obtaining a total count, because they swim too quickly to be reliably counted when alive. The LC_{50} was determined using Finney's probit analysis method (31), with an extract having an LC_{50} of $\leq$1000.0 μg/ml being considered active (15).

Extracts, fractions, and isolates were assayed for cytotoxic activity against the in vitro P-388 murine lymphocytic leukemia bioassay according to the procedures of the National Cancer Institute (16). A crude extract with an ED_{50} of $\leq$20.0 μg/ml or a pure compound with an ED_{50} of $\leq$4.0 μg/ml was considered to be active (16). Coprecipitation of minquartynoic acid with PVP did not significantly alter the observed P-388 ED_{50}.

EXTRACTION AND ISOLATION.—The powdered plant material (1.0 kg) was extracted successively by room-temperature percolation with petroleum ether (bp 40–60°), $CHCl_3$, MeOH, and 50% aqueous EtOH (2 liters, 3×); extracts were evaporated to dryness in a rotary evaporator at 40° or lyophilized and stored in brown glass at 0°.

The $CHCl_3$ extract (68.9 g) was the most biologically active, with a P-388 ED_{50} of 2.16 μg/ml. Cc (open column, 8 × 150 cm) over Si gel (1.5 kg, 270–400 mesh) with gradient elution [4 liters each of $CHCl_3$ 100%, then $CHCl_3$ with EtOAc and MeOH each 1%, then 2%, 5%, 10%, and a final wash with $CHCl_3$-MeOH (1:1)] of the $CHCl_3$ extract yielded 12 fractions which were subjected to the P-388 bioassay. The most active fraction (13.0 g, ED_{50} 0.64 μg/ml) was then subjected to vacuum liquid chromatography [8 × 40 cm column, 1 kg Si gel 70–230 mesh, eluted with $CHCl_3$-EtOAc-MeOH (18:1:1)] which yielded 10 fractions; these fractions were subjected to the P-388 bioassay. From the most active of these fractions (7.0 g, ED_{50} 0.36 μg/ml), 100 mg was dissolved in boiling CH_2Cl_2 (sufficient to give a saturated solution) and allowed to cool gradually, to crystallize 92.6 mg of pure compound with a P-388 ED_{50} of 0.18 μg/ml. The estimated total yield of minquartynoic acid is thus 0.6%.

Minquartynoic acid [**1**].—Gray-yellow needle-like crystals from CH_2Cl_2, decomposes on exposure to light, air, and temperatures over 100°; mp 95°; $[\alpha]^{25}D$ −29° (c = 0.1, MeOH); uv λ max (MeOH) (log ε) 239 (5.46), 227 (5.33), 217 (4.96), 208 (4.63) nm; cd λ max (10 μg/ml, MeOH) 240, 230 nm, no Cotton effect; ir ν max (KBr) 3350, 3300–2500 (broad), 2221, 1709, 1406, 1332, 1288, 1244, 1203, 1175, 1091 cm^{-1}; eims (80 eV) *m/z* (rel. int.) $[M]^+$ 284 (20), 267 (23), 266 (94), 251 (5), 237 (4), 223 (11), 211 (21), 197 (31), 179 (72), 165 (77), 153 (72), 141 (63), 127 (65), 115 (61), 99 (30), 87 (38), 77 (29), 55 (31), 43 (100); eims (20 eV) *m/z* $[M]^+$ 284 (31), 266 (100); negative ion cims *m/z* 286 (3), 285 (21), $[M]^-$ 284 (100), 266 (4), 240 (8); fabms *m/z* $[M - H + 2Li]^+$ 297 (84), $[M + Li]^+$ 291 (82), 105 (100); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

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