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NOVEL ACETYLENIC ACIDS FROM THE ROOT BARK OF *PARAMACROLOBIUM CAERULEUM*: INHIBITORS OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE

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ABSTRACT.—The *n*-hexane extract of the root bark of *Paramacrolobium caeruleum*, collected in Kenya, yielded eleven long chain fatty acids, of which acids **1a**, **2a**, **6a**, **7a**, **9a**, and **10a** are novel. These acetylenic acids are among the first natural products isolated from plants that have been shown to inhibit the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme responsible for the formation of mevalonate in the rate-determining step of cholesterol biosynthesis.

The leading cause of death in the Western world is coronary artery disease, and there is considerable evidence in support of a direct correlation between the incidence of this widespread disorder and elevated levels of plasma cholesterol (1,2). As a large proportion of the total cholesterol in the body is accounted for by de novo synthesis (3) in the liver and intestine, the search for drugs to control cholesterol biosynthesis has long been pursued as a means of regulating plasma cholesterol levels. The major rate-limiting step in the biosynthesis of cholesterol is the reduction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate, which is catalyzed by the enzyme HMG-CoA reductase (4). Considerable success has been achieved in the search for naturally occurring inhibitors of HMG-CoA reductase from microbial sources. In a study of 8000 strains of microorganisms (5), two potent, low toxicity inhibitors, ML-236B (compactin) and monacolin K, were discovered in the cultured broth of the fungi *Penicillium citrinum* (6) and *Monascus ruber* (7), respectively. Monacolin K is identical to mevinolin, which was isolated independently (8) from another fungus, *Aspergillus terreus*, and shown to be a very potent inhibitor of HMG-CoA reductase (K_i of 0.6 nm).

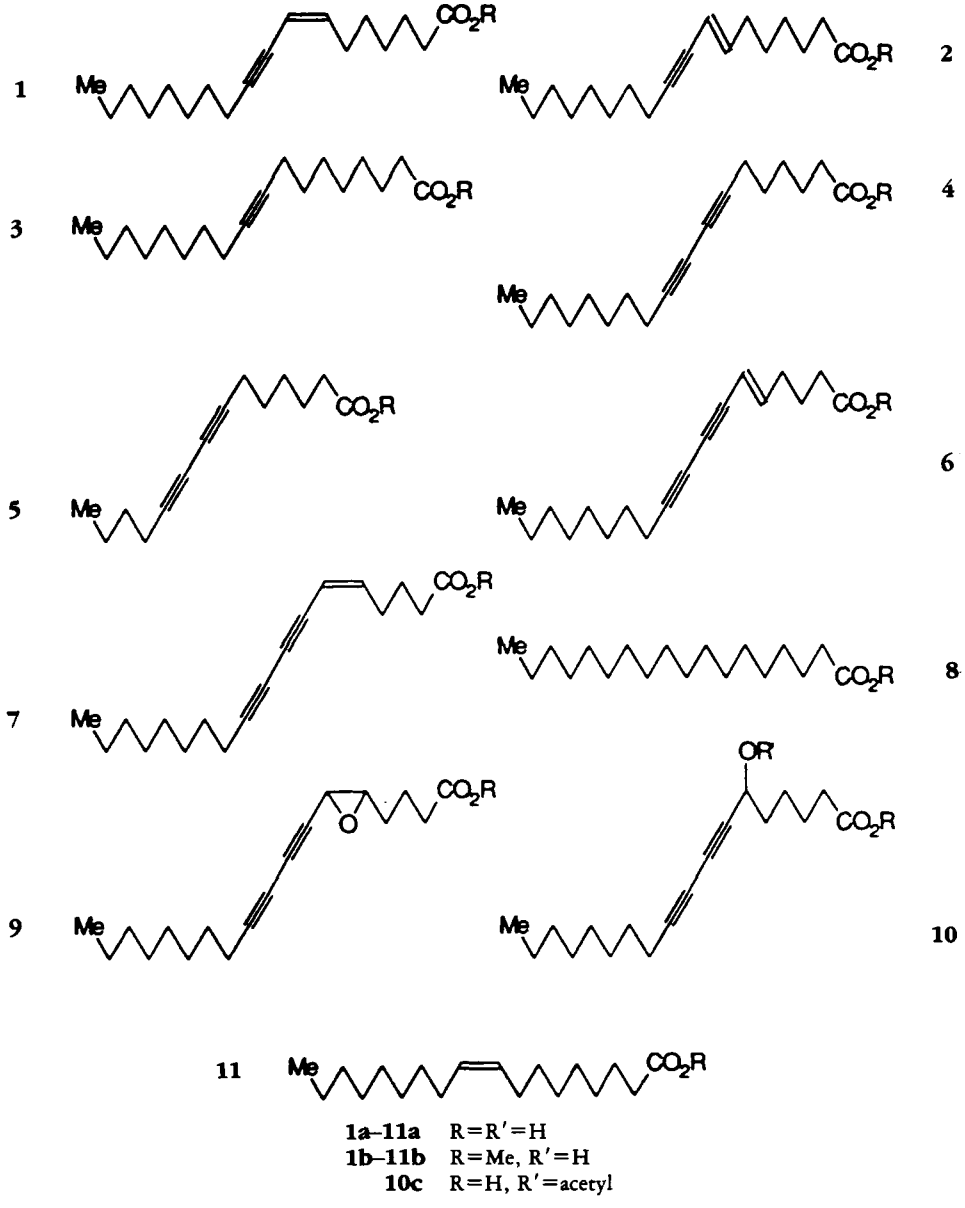
As part of a feasibility study, a search for inhibitors of HMG-CoA reductase in terrestrial plants was carried out on approximately 600 plant extracts using a bioassay-directed screen with enzyme isolated from rat liver (9,17). Ten extracts were found with IC_{50} values less than 100 ug/ml and one of these, the hexane extract of the Kenyan plant *Paramacrolobium caeruleum* (Taub) Leonard (Fabaceae), was selected for fractionation; the results are presented in this paper.

RESULTS AND DISCUSSION

Reversed-phase (C_{18}) Si gel column chromatography of the *n*-hexane extract of the root bark of *P. caeruleum* afforded several fractions showing substantial activity in HMG-CoA reductase (HMGR) assays. These fractions after preparative thin-layer chromatography (ptlc) and reversed-phase high performance liquid chromatography (rp-hplc) provided ten straight chain C_{18} acids, including oleic and stearic, and a C_{14} acid. The eleven identified acids (Figure 1) are: (*Z*)-7-octadecen-9-ynoic [**1a**], (*E*)-7-octadecen-9-ynoic [**2a**], 9-octadecynoic [**3a**], 7,9-octadecadiynoic [**4a**], 7,9-tetradecadiynoic [**5a**], (*E*)-5-octadecen-7,9-diynoic [**6a**], (*Z*)-5-octadecen-7,9-diynoic [**7a**], stearic [**8a**], 3-(1,3-dodecadiynyl)-6-oxiranebutanoic [**9a**], 6-hydroxy-7,9-octadecadiynoic [**10a**], and (*Z*)-9-octadecenoic(oleic) acid [**11a**].

A molecular formula of $C_{18}H_{30}O_2$ was established for **1a** by lreims, which gave a molecular ion at *m/z* 278 and hreims data (278.2245; calcd for $C_{18}H_{30}O_2$, 278.2246).

The ir spectrum indicated a carboxylic acid (1710 cm^{-1}) and a disubstituted acetylenic group (2210 cm^{-1}); the uv spectrum showed absorption (227 nm) characteristic of a conjugated diene or enyne group. The ^{1}H-nmr ($CDCl_3$) spectrum displayed a triplet at δ 2.36 (2H) and quintet (2H) at δ 1.64 assigned to the 1- and 2-CH_2 groups and a triplet (2H) at δ 2.33 due to a methylene adjacent to an acetylenic bond. A broad doublet at δ 5.42 (J = 10.6 Hz) and a doubled triplet at δ 5.82 (10.6 and 7.0 Hz) represent the olefinic protons of a (*Z*)-disubstituted double bond. The ^{13}C-nmr spectrum contained signals indicative of a disubstituted acetylene (δ 77.6, 94.3, s), a double bond (δ 109.2, 142.7, d) and a carbonyl (δ 180.3, s), which accounted for all of the unsaturation in **1a**. Esterification (CH_2N_2) gave a more stable ester **1b** ($C_{19}H_{32}O_2$, hreims), the



1a–11a R=R′=H
1b–11b R=Me, R′=H
10c R=H, R′=acetyl

FIGURE 1. Structures of the eleven acids **1a–11a** isolated from *Paramacrolobium caeruleum* and their synthetic methylesters **1b–11b**. The acetyl derivative **10c** of acid **10a** was also prepared.

TABLE 1. ^{13}C Chemical Shifts for Compounds **1a**, **2a**, **3a**, **4a**, **5a**, **6b**, **7b**, **9b**, and **10b** as determined in $CDCl_3$ by DEPT analysis.

Carbon	Compound								
	1a	**2a**	**3a**	**4a**	**5a**	**6b**	**7b**	**9b**	**10b**
1	180.3 s	180.7 s	180.7 s	180.3 s	181.3 s	174.3 s	174.3 s	174.3 s	174.3 s
2	31.7 t	32.9 t	34.1 t	34.1 t	34.0 t	34.1 t	34.0 t	34.0 t	34.0 t
3	24.6 t	24.7 t	24.9 t	24.9 t	24.0 t	24.9 t	24.8 t	24.9 t	24.8 t
4	28.3[a] t	28.7[a] t	29.7[a] t	28.2[a] t	28.3[a] t	28.2[a] t	28.1[a] t	28.7[a] t	24.5 t
5	28.8[a] t	28.7[a] t	29.2[a] t	28.5[a] t	28.6[a] t	148.3 d	147.8 d	45.5 d	37.8 t
6	28.9[a] t	28.8[a] t	29.1[a] t	19.2 t	19.2 t	108.6 d	108.6 d	60.9 d	62.8 d
7	142.7 d	143.5 d	29.1[a] t	65.3[b] s	65.3[b] s	65.3[b] s	65.2[b] s	81.3[b] s	77.3[a] s
8	109.2 d	109.7 d	18.8 t	77.6[b] s	77.6[b] s	74.1[b] s	78.1[b] s	64.5[b] s	69.9[a] s
9	77.6[b] s	79.3[b] s	80.1[b] s	65.2[b] s	65.1[b] s	72.8[b] s	72.1[b] s	72.4[b] s	81.5[a] s
10	94.3[b] s	88.5[b] s	80.3[b] s	77.3[b] s	77.3[b] s	83.5[b] s	84.7[b] s	68.7[b] s	64.4[a] s
11	19.5 t	19.3 d	18.8 t	19.2 t	19.2 t	19.5 t	19.6 t	19.0 t	19.1 t
12	28.7[a] t	28.9[a] t	29.1[a] t	28.8[a] t	28.5[a] t	28.2[a] t	28.7[a] t	28.9[a] t	28.5[b] t
13	28.7[a] t	28.5[a] t	28.0[a] t	28.6[a] t	22.5 t	28.6[a] t	28.7[a] t	28.6[a] t	27.9[b] t
14	28.7[a] t	28.5[a] t	28.8[a] t	28.7[a] t	14.0 q	28.8[a] t	28.9[a] t	28.9[a] t	28.0[b] t
15	29.0[a] t	28.6[a] t	28.8[a] t	28.9[a] t	—	28.9[a] t	30.4[a] t	27.6[a] t	28.6[b] t
16	30.0 t	32.6 t	31.9 t	31.3 t	—	30.6 t	30.9 t	31.4 t	31.4 t
17	22.8 t	22.6 t	22.8 t	22.5 t	—	22.1 t	22.3 t	22.4 t	22.5 t
18	14.1 q	14.0 q	14.0 q	14.1 q	—	13.8 q	13.9 q	13.9 q	13.9 q
OMe	—	—	—	—	—	51.5 q	51.5 q	51.5 q	51.4 q

[a,b]Values in the same column with the same superscript may be interchanged.

ir spectrum (1743 cm^{-1}) of which indicated a saturated ester. Characterization of **1a** as (*Z*)-7-octadecen-9-ynoic acid was based mostly on the ^1H-nmr spectrum of **1b** in C_6D_6 (Table 2) and extensive decoupling experiments. Irradiation of the 4H multiplet at δ 1.14–1.18, assigned to 4- and 5-CH_2 groups, caused the 3-CH_2 quintet at δ 1.41 to collapse to a triplet, resulting from its coupling to 2-CH_2, while the allylic -CH_2 multiplet at δ 2.44 collapsed to a broad triplet due to its coupling to the 7-H olefinic proton, establishing the partial structure of **1a** to be -C≡C-CH=CH-$(CH_2)_5$-COOH. Similarly, irradiation of a quintet at δ 1.51, which arises from 12-CH_2, caused the methylene triplet adjacent to acetylene to collapse to a broad singlet confirming the other side of the acetylenic bond to consist of two methylenes.

Acid **2a** is isomeric with **1a** as shown by almost identical spectral characteristics (uv, ir, ^1H nmr) except for the ^1H-nmr signal for the allylic -CH_2, which appeared at a higher field by 0.57 ppm, as well as a doublet at δ 5.62 (15.8 Hz) and a multiplet at δ 6.18 (15.8, 7.1 Hz) indicative of an (*E*) double bond. Esterification (CH_2N_2) of **2a** yielded the methyl ester **2b** ($C_{19}H_{32}O_2$, hreims).

Diacetylenic acid **4a**, the major acetylenic metabolite, was the first to be studied in detail. Its molecular formula was established by hreims as $C_{18}H_{28}O_2$ and the presence of ir bands at 3560–2660, 1711, and a weak band at 2162 cm^{-1} suggested the presence of a carboxylic acid function and acetylenic bonds, respectively, while the uv spectrum (hexane) showed absorptions at 283, 267, 253, 246, and 216 nm, characteristic of a diyne chromophore (10,11). The ^{13}C-nmr spectrum of **4a** exhibited four signals corresponding to quaternary carbons in two conjugated triple bonds at δ 65.3, 65.1, 77.3, and 77.6 ppm (s) and a carbonyl as part of the carboxyl group at δ 180.3 (s). The ^1H-nmr spectrum of **4a** was typical of octadecadiynoic acids (12,13), showing a triplet ($J = 6.9$ Hz) at δ 2.35, a quintet at δ 1.63 assigned to methylenes α and β to the carboxyl group and a triplet ($J = 6.7$ Hz) at 0.89 due to the terminal methyl. A 4H triplet ($J = 7.0$ Hz) at δ 2.25 arose from two methylenes adjacent to acetylene units. Conclusive evidence regarding the location of the conjugated acetylene group in the carbon chain of **4a** was obtained from a 2D ^1H-nmr [COSY(14)] spectrum and was also sup-

TABLE 2. ^{1}H-Nmr Data for Compounds **1b**, **2b**, **3b**, **4a**, **5a**, **6b**, **7b**, **9b**, and **10a** in $CDCl_3$ (400 MHz).

Proton	Compound								
	1b[a]	**2b**[a]	**3b**	**4a**	**5a**	**6b**	**7b**	**9b**	**10a**
1	—	—	—	—	8.79 (br, 1H, D_2O)	—	—	—	8.35 (br, 1H, D_2O)
2	2.21 (t, 2H, J = 6.9 Hz)	2.20 (t, 2H, J = 6.9 Hz)	2.30 (t, 2H, J = 7.4 Hz)	2.35 (t, 2H, J = 6.9 Hz)	2.38 (t, 2H, J = 6.9 Hz)	2.30 (t, 2H, J = 7.4 Hz)	2.35 (t, 2H, J = 7.0 Hz)	2.31 (t, 2H, J = 7 Hz)	2.25 (t, 2H, J = 6.9 Hz)
3	1.41 (quint, 2H)	1.40 (quint, 2H)	1.60 (m, 2H)	1.63 (m, 2H)	1.63 (bt, 2H)	1.61 (m, 2H)	1.64 (m, 2H)	1.62 (m, 2H)	1.65 (m, 2H)
4	—	1.31 (m, 2H)	—	1.34 (m, 2H)	1.33 (m, 2H)	2.13 (m, 2H)	2.32 (m, 2H)	1.54 (m, 2H)	1.54 (m, 2H)
5	1.14–1.18 (m, 4H)	1.25 (m, 2H)	—	1.52 (quint, 2H)	1.53 (quint, 2H)	6.27 (dt, 1H, J = 15.8, 7.2 Hz)	6.04 (dt, 1H, J = 10.8, 7.5 Hz)	3.08 (m, 1H)	1.38 (m, 2H)
6	2.44 (m, 2H)	1.87 (m, 2H)	1.25–1.41 (m)	2.25 (t, 2H, J = 7 Hz)	2.24 (t, 2H, J = 6.9 Hz)	5.48 (d, 1H, J = 15.8 Hz)	5.46 (d, 1H, J = 10.8 Hz)	3.12 (d, 1H, J = 1.1 Hz)	4.35 (t, 1H, J = 6.8 Hz)
7	5.69 (dt, J = 10.8, 7.2 Hz)	6.18 (dt, 1H, J = 15.8, 7.1 Hz)	—	—	—	—	—	—	—
8	5.60 (d, J = 10.8 Hz)	5.62 (d, 1H, J = 15.8 Hz)	2.13 (t, 2H, J = 7 Hz)	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—
11	2.08 (t, 2H, J = 7.2 Hz)	2.07 (t, 2H, J = 7.4 Hz)	2.13 (t, 2H, J = 7 Hz)	2.25 (t, 2H, J = 7 Hz)	2.25 (t, 2H, J = 6.9 Hz)	2.31 (t, 2H, J = 7.4 Hz)	2.31 (t, 2H, J = 7.4 Hz)	2.27 (t, 2H, J = 6.9 Hz)	2.20 (t, 2H, J = 7.0 Hz)
12	1.51 (quint, 2H)	1.49 (quint, 2H)	—	1.52 (quint, 2H)	1.53 (quint, 2H)	1.53 (quint, 2H)	1.55 (quint, 2H)	1.54 (m, 2H)	1.51 (m, 2H)
13	—	—	—	—	1.28–1.45 (m, 2H)	—	—	—	—
14	—	—	1.25–1.42 (m)	—	—	1.25–1.42 (m, 10H)	—	—	—
15	1.19–1.30 (m, 10H)	1.05–1.21 (m, 10H)	—	1.20–1.40 (m, 10H)	0.89 (t, 3H, J = 6.8 Hz)	—	1.22–1.44 (m, 10H)	1.20–1.45 (m, 10H)	1.22–1.45 (m, 10H)
16	—	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—	—
18	0.84 (t, 3H, J = 7.1 Hz)	0.85 (t, 3H, J = 7 Hz)	0.88 (t, 3H, J = 6.6 Hz)	0.89 (t, 3H, J = 6.7 Hz)	—	0.89 (t, 3H, J = 7 Hz)	0.89 (t, 3H, J = 7.1 Hz)	0.89 (t, 3H, J = 7.2 Hz)	0.90 (t, 3H, J = 7.1 Hz)
OMe	3.35 (s, 3H)	3.35 (s, 3H)	3.67 (s, 3H)	—	—	3.67 (s, 3H)	3.67 (s, 3H)	3.68 (s, 3H)	—

[a] C_6H_6-d_6

ported by decoupling experiments. The spectrum indicated the presence of coupling between 2-CH_2 (δ 2.35) and 3-CH_2 (δ 1.63), which was in turn coupled to the 4-CH_2 (δ 1.34). Similarly, the 6,11-methylene triplets (δ 2.25) were coupled to the 5,12-methylene quintets (δ 1.52), thus establishing the structure of **4a** as 7,9-octadecadiynoic acid.

Treatment of **4a** with ethereal CH_2N_2 yielded the methyl ester **4b** with a molecular ion at *m/z* 290 $[M]^+$ (lreims) and the molecular formula $C_{19}H_{30}O_2$ as indicated by hreims. The ir (film) spectrum had the absorption at 1741 cm^{-1} expected for an ester carbonyl group. The ^{1}H-nmr spectrum showed a methoxyl singlet at δ 3.68, while the remainder of the protons showed signals at virtually identical positions to those found for **4a**. The ^{13}C-nmr spectrum of **4b** is also consistent with the assigned structure, and its DEPT analysis confirmed the number and type of protonated and quaternary carbons.

With the structure of acid **4a** in hand, the structures of the closely related acids **5a**, **6a**, **7a**, **9a**, and **10a** were readily determined. Compound **5a** ($C_{14}H_{20}O_2$, hreims) was shown to be a lower homologue of **4a** with very similar ir, uv, ^{13}C-nmr, and ^{1}H-nmr spectra, except for the broad absorption at δ 1.28–1.45 that integrated for eight fewer hydrogens, reflecting a shorter alkyl chain. In addition, ^{13}C-nmr signals for fourteen carbons of **5a** appeared at positions identical to those in the spectrum of **4a** except for the missing methylenes in the aliphatic chain. Although compounds **4a** and **5a** have been synthesized (15,16), this represents the first reported isolation of these acids from natural sources.

The molecular ion for **6a** at *m/z* 275 $[M+H]$ had the elemental composition $C_{18}H_{26}O_2$, two hydrogens fewer than **4a**. The uv and ir spectra of **6a** were similar to **4a** suggesting acetylenic and carboxylic acid groups. The ^{1}H nmr showed signals for two methylenes α and β to the carboxyl group at δ 2.30 and 1.61, respectively, and one methylene group at δ 2.31 adjacent to an acetylene moiety. A broad doublet at δ 5.48 (J = 15.8 Hz) and a doubled triplet at δ 6.27 (15.8, 7.2 Hz), each of which was converted to a sharp doublet by irradiation in the allylic region (δ 2.13), provided evidence for a -C≡C-C≡C-CH=CH-CH_2-unit with an (*E*) double bond configuration. The ^{13}C-nmr spectrum displayed signals at δ 108.6 and 148.3 (both d) due to carbons C-5 and C-6, and DEPT analysis confirmed the number and substitution pattern of carbon atoms in **6a**. Esterification of **6a** (CH_2N_2) gave a pure methyl ester **6b**. The uv spectra of **6a** and **6b** were identical, and the 400 MHz ^{1}H-nmr spectrum of **6b** had a singlet at δ 3.67 (3H) due to the methyl ester group.

Acid **7a** is isomeric with **6a**, and this is reflected in the very similar spectral characteristics (uv, ir, ^{1}H nmr) of the two acids. One exception was the ^{1}H-nmr signal in **7a** due to the allylic -CH_2, which was observed at 0.2 ppm lower field (δ 2.32). The major difference was the olefinic proton signals; a sharp doublet in the spectrum of **7a** at δ 5.46 (10.8 Hz) and a multiplet at 6.04 (10.8, 7.5 Hz) indicated a (*Z*) double bond. Esterification of **7a** with CH_2N_2 gave a methyl ester ($C_{19}H_{28}O_2$, hreims).

The novel epoxide **9a** obtained as an unstable yellow gum was assigned the indicated structure on the basis of the following evidence. The lr and hreims ($[M]^+$ 290) supported a molecular formula of $C_{18}H_{28}O_3$. The ir, uv, ^{1}H-nmr, and ^{13}C-nmr spectra were very similar to those of the related acids with a few notable exceptions, and in particular the additional unsaturation in the chain of **9a** was identified as being due to an epoxide moiety. The ^{1}H-nmr spectrum of **9a** closely paralleled that of **6a**, with the assignments again based on the homonuclear 2D nmr spectrum. The major difference was the replacement of the vinylic protons at δ 5.48 and 6.27 with carbinol protons at δ 3.08 (m, 1H) and 3.12 (d, 1H, J = 1.1 Hz). The ^{13}C nmr of **9a** displayed all 18 carbons and closely resembled the spectrum of **6a**, except for the replacement by two

oxygenated methine carbons [δ 45.4 and 60.8 (both d)] for two olefinic carbon signals at δ 108.6 and 148.3. DEPT analysis of the ^{13}C-nmr spectrum of **9a** showed five quaternary, two methine, ten methylene, and one methyl carbon. Treatment of **9a** with CH_2N_2 provided its methyl ester **9b** ($C_{19}H_{28}O_3$, hreims), whose ^{1}H nmr had a methoxyl singlet (3H) at δ 3.68.

Another novel compound isolated as a minor metabolite displayed HMGR activity and was identified as 6-hydroxy-7,9-octadecadiynoic acid [**10a**]. In this case, lreims showed a molecular ion at *m/z* 292, while the elemental composition $C_{18}H_{28}O_3$ was deduced by hreims and was supported by ^{13}C-nmr data. The acid was isolated as a colorless solid, mp 47–49°, and exhibited ir bands at 3500–3000, 2235, and 1709 cm^{-1}, indicating carboxyl and acetylene functions. A ^{13}C-nmr signal at δ 62.8 (d) supported the presence of an oxygenated methine carbon in **10a**. Exchangeable proton signals were observed in the ^{1}H-nmr spectrum at δ 8.35 and 2.01, consistent with the presence of carboxyl and hydroxyl groups. The H-6 proton appeared as a triplet at δ 1.38. On irradiation of this multiplet, the triplet collapsed to a singlet. Reaction of **10a** with CH_2N_2 gave an oily methyl ester **10b** that showed ir absorption at 3450, 2233, and 1740 cm^{-1} indicating the presence of a hydroxyl group in addition to acetylene and ester groups and uv maxima identical with those of **10a**. Acetylation (Ac_2O/C_5H_5N) of **10a** yielded a monoacetate **10c**, whose ^{1}H-nmr spectrum contained an acetyl methyl signal at δ 2.15 (s, 3H) while the rest of the protons showed resonances at virtually identical positions except for the methine proton (δ 5.09), which was shifted downfield.

The acids **3a**, **8a**, and **11a** were readily identified by comparison of their spectral characteristics, gc and gc-ms data, and in some cases by direct comparison with authentic samples.

BIOACTIVITY OF THE ACETYLENIC ACIDS.—Assays for inhibition of HMGR activity were performed as previously reported (8,17) using a purified catalytic fragment of human HMGR (18). The assay conditions were as reported except for the concentrations of HMG-CoA, NADPH, and enzyme, which were 10 μM, 200 μM, and 20 pM, respectively. The isolated natural products were dissolved at 5 mg/ml in 0.1 N NaOH, and dilutions were made in 10 mM potassium phosphate, pH 7.2. The stock solutions were shown to be stable with respect to decomposition over the time period of the assay. Concentrations giving 50% inhibition were determined using at least 5 concentrations of inhibitors and were reproducible to approximately 50% between different preparations of acetylenic acids with the exception of compound **9a** which proved to be very unstable (Table 3).

For comparison, the fungal metabolite mevinolin, which is the most potent known inhibitor for this enzyme, has been shown to have an IC_{50} of 2 nm in the assay described above. The inhibition observed for the acetylenic acids is, therefore, quite modest but intriguing because of their structural dissimilarity to other known inhibitors of HMGR.

For (*E*)-7-octadecen-9-ynoic acid [**2a**] (0.2, 0.4, and 0.8 μg/ml) and (*E*)-5-octadecen-7,9-diynoic acid [**6a**] (0.01, 0.03, and 0.06 μg/ml), double reciprocal plots for 1/v vs. 1/[HMG-CoA] were generated using appropriate concentrations of inhibitor, based on IC_{50}. The resulting kinetic data, however, did not seem to fit any of the classical inhibitor models (competitive, uncompetitive, and noncompetitive) suggesting that the effect of these acids is a nonspecific inhibition possibly of a detergent type.

Acetylenic acids are well-known constituents of terrestrial plants (19), but to our knowledge no such acids have been tested in HMGR assays. This represents the first report of acetylenic acids exhibiting HMGR activity.

TABLE 3. Inhibition of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase by Acetylenic Acids Isolated from *Paramacrolobium caeruleum*.

Acid	IC_{50}	
	microgram/ml	micromolar
(*Z*)-7-Octadecen-9-ynoic [**1a**]	0.9	3
(*E*)-7-Octadecen-9-ynoic [**2a**]	0.6	2
9-Octadecynoic [**3a**]	1.5	5
7,9-Octadecadiynoic [**4a**]	1.5	5
7,9-Tetradecadiynoic [**5a**]	0.6	3
(*E*)-5-Octadecen-7,9-diynoic [**6a**]	0.15	0.5
(*Z*)-5-Octadecen-7,9-diynoic [**7a**]	0.4	1.5
Octadecanoic (stearic) [**8a**]	no inhibition	
3-(1,3 dodecadiynyl)-6-Oxiranebutanoic [**9a**]	1.5	5
6-Hydroxy-7,9-octadecadiynoic [**10a**]	2	7
(*Z*)-9-Octadecenoic(oleic) [**11a**]	no inhibition	

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. ^{1}H-, ^{13}C-, and 2D-nmr spectra were obtained on a Bruker AM-400 spectrometer, and chemical shifts (δ) were reported in parts per million downfield from internal TMS standard. Gc-ms and lreims were performed on a Finnigan 4610 instrument, and hreims on a Finnigan MAT 731 spectrometer. Analytical and preparative tlc were carried out on precoated Si gel G (Kieselgel G254) and reversed-phase (Whatman KC18F) plates. A Beckman 114M solvent delivery system equipped with a refractive index detector, Model 156, was used for hplc on Whatman Partsil 10, ODS-3 (Magnum-9) column. Uv spectra were recorded on a Beckman DU-7 spectrophotometer. Reagent grade chemicals (Fisher and Baker) were used. Gc analysis was carried out using a Hewlett Packard 5790A series gas chromatograph.

ISOLATION OF COMPOUNDS **1a–11a** FROM *P. CAERULEUM*.—*P. caeruleum* was collected in Kenya in September 1980 and was identified by J. Leonard (National Cancer Institute). A voucher specimen SS-1566 is preserved at the National Herbarium, Washington, D.C. The root bark (400 g) was extracted three times for 7-day periods with *n*-hexane by cold percolation procedure. The residue (2.2 g) obtained after evaporation of the solvent exhibited activity in HMGR inhibitor assays. Si gel tlc [MeOH-CH_2Cl_2 (5:95)] of the *n*-hexane extract suggested the presence of 5 or 6 compounds, but rp-tlc [H_2O-MeCN (30:70)] indicated it contained at least 13 or 14 compounds. Cc of the residue (2.05 g) from the *n*-hexane extract [Whatman RP-18, 40 g, H_2O-MeCN, (20:80)] was carried out. A total of 184 fractions (6–7 ml) were collected and pooled according to their tlc behavior to give 8 bioactive fractions. These fractions, after exhaustively repeated ptlc and rp-hplc [Whatman column, H_2O-MeCN (1:4)] employing refractive index detector, provided 11 pure acids: **1a** (23 mg), **2a** (14 mg), **3a** (11 mg), **4a** (128 mg), **5a** (6 mg), **6a** (42 mg), **7a** (21 mg), **8a** (103 mg), **9a** (16 mg), **10a** (18 mg), and **11a** (663 mg) in order of elution.

(*Z*)-7-OCTADECEN-9-YNOIC ACID [**1a**].—Low melting, colorless solid, ir (CCl_4) 3440–3000, 2856, 2210, 1710, 1461, 1249 cm^{-1}; λ max (hexane) 227 nm; ^{1}H nmr ($CDCl_3$) δ 10.42 (br, 1H, D_2O), 5.82 (dt, 1H, J = 10.6, 7.4 Hz); 5.42 (d, 1H, J = 10.6 Hz), 2.36 (t, 2H, J = 6.9 Hz), 2.33 (t, 2H, J = 7.1 Hz), 2.27 (m, 2H), 1.64 (quint, 2H), 1.54 (quint, 2H), 1.20–1.45 (m, 14H), 0.89 (t, 3H, J = 6.9 Hz), ^{13}C nmr see Table 1; lreims *m/z* [M]$^+$ 278, 261, 251, 219, 185, 99, 88, 73. *Anal.* calcd for $C_{18}H_{30}O_2$, *m/z* 278.2246; found 278.2245.

(*Z*)-7-OCTADECEN-9-YNOIC ACID METHYL ESTER [**1b**].—An oil, ir (film) 2860, 2211, 1743, 1463, 1249 cm^{-1}; λ max (hexane) 228 nm; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; lreims *m/z* [M]$^+$ 292, 261, 243, 219, 150, 93, 79, 67. *Anal.* calcd for $C_{19}H_{32}O_2$, *m/z* 292.2402; found 292.2396.

(*E*)-7-OCTADECEN-9-YNOIC ACID [**2a**].—Low melting solid, ir (CCl_4) 3440, 2858, 2216, 1709, 1412, 954 cm^{-1}; λ max (hexane) 228 nm; ^{1}H nmr ($CDCl_3$) δ 8.73 (br, 1H, D_2O), 6.03 (dt, 1H, J = 15.9, 7.2 Hz), 5.46 (d, 1H, J = 15.9 Hz), 2.39 (m, 2H), 2.29 (t, 2H, J = 6.9 Hz), 2.06 (m, 2H), 1.62 (m, 2H), 1.53 (quint, 2H), 1.21–1.42 (m, 14H), 0.89 (t, 3H, J = 7.1 Hz); ^{13}C nmr see Table 1; lreims *m/z* [M]$^+$ 278, 261, 251, 219, 185, 99, 88, 73.

(*E*)-7-OCTADECEN-9-YNOIC ACID METHYL ESTER [**2b**].—Colorless oil, ir (film) 2860, 2214,

1740, 1419, 960 cm^{-1}; λ max (hexane) 229 nm; ^1H nmr see Table 2; lreims *m/z* $[M]^+$ 292, 261, 150, 93, 79, 67, 55, 41. *Anal.* calcd for $C_{19}H_{32}O_2$, *m/z* 292.2402; found 292.2398.

7,9-OCTADECADIYNOIC ACID [**4a**].—Colorless powder, mp 41–43°, ir (CCl_4) 3560–2660, 2950, 2162, 1711, 955 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; lreims *m/z* $[M]^+$ 276, 207, 181, 167, 149, 135, 123, 109, 105, 91.

7,9-OCTADECADIYNOIC ACID METHYL ESTER [**4b**].—Colorless oil, ir (film) 2850, 2171, 1741, 950 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.68 (s, 3H), 2.36 (t, 2H, J = 6.8 Hz), 2.25 (t, 4H, J = 7 Hz), 1.63 (quint, 2H), 1.51 (quint, 4H), 1.25–1.46 (m, 12H), 0.89 (t, 3H, J = 6.7 Hz); lreims *m/z* $[M]^+$ 290, 259, 145, 133, 119, 105, 91, 79, 67, 55, 41. *Anal.* calcd for $C_{19}H_{30}O_2$, *m/z* 290.2245; found 290.2245.

7,9-TETRADECADIYNOIC ACID [**5a**].—Colorless gum, ir (CCl_4) 3600–3100, 3000–2800, 2210, 1710, 1243, 944 cm^{-1}; λ max (MeOH) 283, 267, 253, 240, 214 nm; ^1H nmr see Table 2; ^{13}C nmr see Table 1; lreims *m/z* $[M]^+$ 220, 206, 173, 163, 119, 105, 91. *Anal.* calcd for $C_{14}H_{20}O_2$, *m/z* 220.1463; found 220.1461.

7,9-TETRADECADIYNOIC ACID METHYL ESTER [**5b**].—Colorless oil, ir (film) 2800, 2214, 1742, 1245, 941 cm^{-1}; λ max (hexane) 284, 267, 252, 241, 215 nm; ^1H nmr ($CDCl_3$) δ 3.69 (s, 3H), 2.34 (t, 2H, J = 6.9 Hz), 2.24 (t, 4H, J = 7 Hz), 1.63 (quint, 2H), 1.52 (quint, 4H), 1.24–1.48 (1OH, m), 0.89 (t, 3H, J = 6.9 Hz); lreims *m/z* 234.

(*E*)-5-OCTADECEN-7,9-DIYNOIC ACID [**6a**].—Colorless gum, ir (CCl_4) 3435, 2890, 2216, 1709, 945 cm^{-1}; λ max (hexane) 282, 266, 252, 240, 217 nm; ^1H nmr ($CDCl_3$) δ 6.25 (dt, 1H, J = 15.7, 7.2 Hz), 5.47 (d, 1H, J = 15.7 Hz), 2.30 (t, 2H, J = 7.3 Hz), 2.29 (t, 2H, J = 7.3 Hz), 2.12 (m, 2H), 1.63 (m, 2H), 1.51 (m, 2H), 1.25–1.42 (m, 10H), 0.88 (t, 3H, J = 7.1 Hz); lreims *m/z* $[M+H]^+$ 275.

(*E*)-5-OCTADECEN-7,9-DIYNOIC ACID METHYL ESTER [**6b**].—Oil, ir (film) 2895, 2204, 1738, 945 cm^{-1}; λ max (hexane) 282, 267, 252, 240, 218 nm; ^1H nmr see Table 2; ^{13}C nmr see Table 1. *Anal.* calcd for $C_{19}H_{28}O_2$, *m/z* 288.2089; found 288.2093.

(*Z*)-5-OCTADECEN-7,9-DIYNOIC ACID [**7a**].—Colorless gum, ir (CCl_4) 3460–2900, 2885, 2216, 1708, 939 cm^{-1}; λ max (hexane) 284, 268, 253, 241, 217 nm; ^1H nmr ($CDCl_3$) δ 6.05 (dt, 1H, J = 10.7, 7.5 Hz), 5.45 (d, 1H, J = 10.7 Hz), 2.33 (t, 2H, J = 7.0 Hz), 2.32 (m, 2H), 2.30 (t, 2H, J = 7.4 Hz), 1.63 (m, 2H), 1.55 (quint, 2H), 1.20–1.45 (m, 10H), 0.88 (t, 3H, J = 7.0 Hz); lreims *m/z* $[M+H]^+$ 275.

3-(1,3-DODECADIYNYL)-6-OXIRANEBUTANOIC ACID [**9a**].—Yellow gum, ir (CCl_4) 3496–3075, 2930, 2858, 2246, 1461, 1436, 1176, 866 cm^{-1}; λ max (MeOH) 283, 269, 258, 245, 207 nm; ^1H nmr ($CDCl_3$) δ 3.14 (d, 1H, J = 1.1 Hz), 3.10 (m, 1H), 2.30 (t, 2H, J = 7 Hz), 2.26 (t, 2H, J = 7.0 Hz), 1.53 (m, 4H), 1.24–1.48 (m, 10H), 0.89 (t, 3H, J = 7.1 Hz); lreims *m/z* $[M]^+$ 290.

3-(1,3-DODECADIYNYL)-6-OXIRANEBUTANOIC ACID METHYL ESTER [**9b**].—An oil, ir (film) 2933, 2858, 2252, 1739, 1463, 1435, 1172, 879 cm^{-1}; λ max (hexane) 282, 270, 258, 246, 217 nm; ^1H nmr see Table 2; ^{13}C nmr see Table 1; lreims $[M+H]^+$ 305, 273, 162, 117, 105, 91. *Anal.* calcd for $C_{19}H_{28}O_3$, *m/z* 304.2038; found 304.2037.

6-HYDROXY-7,9-OCTADECADIYNOIC ACID [**10a**].—Colorless solid, mp 47–49°, ir (CCl_4) 3500–3000, 2980, 2875, 2235, 1709, 1454, 1436, 1180, 854 cm^{-1}; λ max (MeOH) 266, 258, 239, 229, 215 nm; ^1H nmr see Table 2; lreims *m/z* $[M]^+$ 292.

6-HYDROXY-7,9-OCTADECADIYNOIC ACID METHYL ESTER [**10b**].—An oil, ir (CCl_4) 3450, 2985, 2870, 2233, 1740, 1455, 1440, 1181, 856 cm^{-1}; λ max (hexane) 267, 256, 242, 231, 215 nm; ^1H nmr ($CDCl_3$) δ 4.41 (bt, 1H), 3.68 (s, 3H), 2.28 (t, 2H, J = 7.1 Hz), 2.24 (t, 2H, J = 7.0 Hz), 2.01 (bs, 1H, D_2O exchangeable), 1.70 (m, 2H), 1.63 (m, 2H), 1.53 (m, 2H), 1.20–1.48 (m, 12H), 0.89 (t, 3H, J = 6.9 Hz); lreims *m/z* $[M]^+$ 306, 245, 235, 203, 175, 117, 91, 55, 43. *Anal.* calcd for $C_{19}H_{30}O_3$, *m/z* 306.2194; found 306.2196.

6-ACETOXY-7,9-OCTADECADIYNOIC ACID [**10c**].—Colorless solid, ir (CCl_4) 3500–3000, 2980, 2820, 2234, 1746, 1710, 1456, 1440, 1185, 960 cm^{-1}; λ max (hexane) 267, 258, 243, 232, 216 nm; ^1H nmr ($CDCl_3$) δ 9.0 (br, 1H, D_2O exchangeable), 5.09 (bt, 1H), 2.26 (t, 2H, J = 6.8 Hz), 2.20 (t, 2H, J = 7.0 Hz), 2.15 (s, 3H), 1.65 (m, 2H), 1.55 (m, 2H), 1.52 (m, 2H), 1.19–1.50 (m, 12H), 0.90 (t, 3H, J = 7.0 Hz).

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