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MALYNGIC ACID, A NEW FATTY ACID FROM *LYNGBYA MAJUSCULA*

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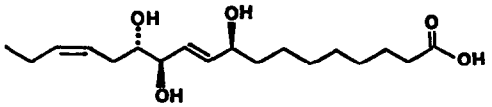
Abstract—Malyngic acid, a major fatty acid in several varieties of the marine blue-green alga *Lyngbya majuscula*, has been determined to be 9(*S*),12(*R*),13(*S*)-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid from chemical and spectral data and by its conversion to 9(*S*),12(*R*),13(*S*)-trihydroxystearic acid and degradation to 2-deoxy-D-ribitol.

The secondary metabolites in shallow-water varieties of the marine blue-green alga *Lyngbya majuscula* are generally different from those found in deep-water varieties. 7(*S*)-Methoxytetradec-4(*E*)-enoic acid, for example, is a constituent of all shallow-water varieties that we have examined so far, but appears to be absent in deep-water varieties.[1] On the other hand, lipids that are related to 7-methoxy-9-methylhexadec-4(*E*)-enoic acid are elaborated by deep-water varieties and these compounds are absent in the shallow-water varieties.[2,3] We have now found that a previously unreported fatty acid is a major constituent in two shallow-water varieties of *L. majuscula* and also a deep-water variety. In this paper we wish to report that this new fatty acid, malyngic acid, is 9(*S*),12(*R*),13(*S*)-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid (**1**).



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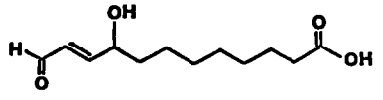
The free fatty acid was obtained by partitioning the methanolic extract of the alga between methylene chloride and water, extracting the aqueous layer exhaustively with ethyl acetate, and subjecting the base-soluble portion of the ethyl acetate extract to liquid chromatography. Malyngic acid was obtained as a waxy solid which melted at 48.5–51.5°.[4] Use of chloroform in the extraction procedure resulted in appreciable esterification of the acid during workup. Generally a mixture of methyl and ethyl malyngate was obtained, the ethyl ester presumably arising from the ethanol (preservative) in the chloroform.

Malyngic acid, a monobasic carboxylic acid with three alcohol groups, formed a methyl ester triacetate derivative which exhibited in its ^{1}H NMR spectrum in $CDCl_3$ a 3 H singlet at δ3.62 for the methoxyl group and 3 H and 6 H singlets at δ1.99 and 2.01 for the three acetoxyl groups. Positive borax and periodate tests indicated the presence of a vicinal diol functionality and malyngic acid readily formed an acetonide derivative. Neither the acid nor any of the aforementioned derivatives produced a molecular ion in the electron-impact MS. The MS of methyl malyngate triacetate, however, did have small fragment ioms for losses of two and three molecules of acetic acid from the molecular ion and the MS of the acetonide showed a M-1 peak. The ^{13}C NMR spectrum of the acid in methanol-d_4 exhibited signals for eighteen carbon atoms, viz one CO carbon, four olefinic methine carbons, three hydroxymethine carbons, nine methylene carbons, and one Me carbon. These data were consistent with the formula $C_{18}H_{32}O_5$ for malyngic acid.

The presence of two 1,2-disubstituted olefinic double bonds were implied from the ^{13}C NMR data. The UV and ^{1}H NMR spectra indicated that the two double bonds were unconjugated and the IR spectrum suggested that one was trans (970 cm^{-1}) and one was *cis* (730 cm^{-1}).

Cleavage of the vicinal diol with periodic acid gave a dextrorotatory hydroxyaldehyde which possessed the *trans* double bond. The ^{1}H NMR spectrum in $CDCl_3$ showed that these three functionalities were present in a γ-hydroxy-α,β-unsaturated aldehyde moiety. The doublet of doublets of doublets at δ6.28 and the doublet of doublets at δ6.82 were assigned to the two olefinic protons and the 16 Hz coupling between them indicated that the double bond was *trans*. The proton at δ6.28 was also vicinally coupled to the aldehydic proton at δ9.57 by 8 Hz and allylically coupled to a OH-bearing methine at δ4.42 by 1.5 Hz. The proton at δ6.82 was further coupled (4.5 Hz) to the proton at δ4.42. The absence of a Me signal and the presence of a triplet at δ2.33 indicated that the carboxyl group was present in this fragment. Mass spectral analysis established $C_{12}H_{20}O_4$ as the molecular formula for the compound. These data showed that the oxidation product had structure **2** and that malyngic acid had partial structure **1a**.



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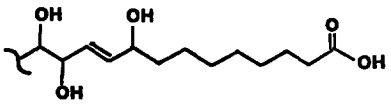
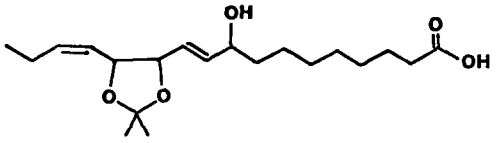


1a

Partial analysis of the ^{1}H NMR spectrum of the acetonide in $CDCl_3$ now allowed us to propose its gross structure as **3**. The presence of a *cis*-2-pentenyl group was concluded from the following spin-spin decoupling experiment. Irradiation of a complex multiplet at δ2.20, which was assigned to the two allylic methylenes (C-14 and C-17), reduced the triplet at δ0.95 for the C-18 Me

protons to a singlet, collapsed the multiplet at δ5.79 for the C-15 and C-16 protons to a broad singlet, and perturbed the 2 H signal at δ4.12 where the C-13 methine proton resonated. The chemical shifts of the two geminal Me groups were quite different (δ1.37 and 1.58), and this meant that the *cis*-2-pentenyl and *trans*-3-hydroxy-10-carboxydec-1-enyl substituents were *cis* to each other on the acetonide ring.



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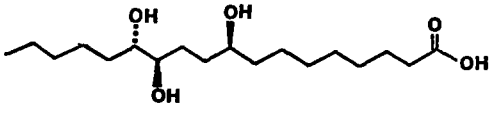
The *cis* geometry of the Δ^{15} double bond was verified by a ^{1}H NMR study of malyngic acid in acetonitrile-d_3 at 220 MHz. The C-15 and C-16 protons were clearly doublets (J = 11 Jz) of triplets (J = 6.5 Hz) at δ5.42 and 5.48, respectively. Irradiation of the quintet at δ2.03, assigned to the methylene protons on C-17 reduced the signal at δ5.48 to a doublet (J = 11 Hz) whereas irradiation of the multiplet at δ2.15, assigned to the methylene protons on C-14, reduced the signal at δ5.42 to a doublet (J = 11 Hz).

On the basis of the data above malyngic acid was an erythro 9,12,13-trihydroxyoctadeca-10(*E*), 15(*Z*)-dienoic acid. To determine the absolute stereochemistry of the triol system, malyngic acid was catalytically hydrogenated. The resulting tetrahydromalyngic acid proved to be identical with 9(*S*),12(*R*),13(*S*)-trihydroxystearic acid (**4**), one of the two erythro isomers obtained by the *cis* hydroxylation of 9(*S*)-hydroxyoctadec-12(*Z*)-enoic acid (**5**), a constituent of *Strophanthus* seed oils, with permanganate.[5] The two



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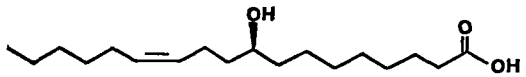
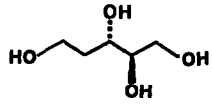


5

acids had the same R_f values when compared directly by tlc and exhibited the same ^{13}C NMR spectra. The chromatographic and spectral properties of 9(*S*),12(*S*),13(*R*)-trihydroxystearic acid, the other erythro isomer produced from **5**, were completely different. Tetrahydromalyngic acid, which appeared to be polymorphic, had m.ps that were close to values reported in the literature for **4** and its optical rotation was similar to that of **4** by direct comparison (Table 1). The structure of malyngic acid was therefore **1**.

Conclusive proof of the absolute stereochemistry at C-12 and C-13 was obtained by ozonolysis (reductive workup) of malyngic acid to 2-deoxy-D-ribitol (**6**).



6

EXPERIMENTAL

^{1}H NMR spectra were obtained at 100 and 220 MHz and ^{13}C NMR spectra were obtained at 25 MHz. ^{1}H chemical shifts are reported in δ units (ppm) relative to TMS (δ = 0) and ^{13}C chemical shifts are reported in δ units relative to methanol-d_4 (δ = 48.3) or dimethyl sulfoxide-d_6 (δ 39.6) as internal standards. Optical rotations were determined on an automatic polarimeter. M.ps are uncorrected. MS were obtained at 70 eV.

Isolation of malyngic acid (**1**). *Lyngbya majuscula* was collected in shallow water at Bruce Island, Enewetak Atoll in February 1976, and was kept frozen until processing. The wet alga (4 kg) was extracted successively with MeOH and CH_2Cl_2. The methanolic extract was concentrated *in vacuo* and the aqueous concentrate was washed with the CH_2Cl_2 extract. The aqueous phase was then exhaustively extracted with EtOAc. The EtOAc extract was evaporated to give 4.3 g of a brown gum. A portion

Table 1. Comparison of melting points and optical rotations of tetrahydromalyngic acid and the four diastereomeric 9(*S*),12,13-trihydroxystearic acids

	M.p.	$[\alpha]_D$
Tetrahydromalyngic acid	105-108.5° 108.5-112° 112.5-115.5° 114.5-122.5°	-5.8° (MeOH)
9(S),12(R),13(S)-Trihydroxystearic acid	101-103° [b] 102-105° [c] 108-110° [d] 112-119° [e]	-3.3° (MeOH)[a] +0.1° (HOAc)[c]
9(S),12(S),13(R)-Trihydroxystearic acid	148-150° [b,c]	+5.5° (HOAc)[c]
9(S),12(R),13(R)-Trihydroxystearic acid	87-89° [b] 89.5-90.5°[c]	+28.5° (HOAc)[c]
9(S),12(S),13(S)-Trihydroxystearic acid	oil[b]	levorotatory in HOAc[c]

[a]This work. [b]Ref. 5. [c]Ref. 6. [d]Ref. 7. [e]Ref. 8.

(1 g) of this gum was partitioned between 5% aq NaOH and EtOAc, the aqueous phase was decolorized woth charcoal, acidified, and extracted with EtOAc, and the EtOAc extract was dried and evaporated to give 880 mg of a tan oil. Further purification was achieved by HPLC on a column of Whatman MAG-9 ODS-2 using MeOH-1% aqueous HOAc (7:3) as the eluant to give 366 mg of a colorless oil which crystallized at 5°. Trituration with cold hexane gave **1** as a waxy solid, m.p. 48.5–51°, $[\alpha]_d^{24.5}$ + 7.5° (*c*1.2,MeOH), + 3.3°(*c*1.6,acetone); IR(neat) 3400, 1715, 970, 730 cm^{-1}; ^1H NMR(CD_3CN) δ5.67(2 H on C-10 and C-11,m), 5.48(1 H on C-16,dt,J = 11 and 6.5 Hz), 5.42(1 H on C-15,dt,J = 11 and 6.5 Hz), 4.7–3.6(br,3 OH), 3.97(1 H on C-9 or C-12,br m), 3.93(1 Hon C-9 or C-12,br m), 3.52(1 H on C-13,dt,J = 8 and 5.5 Hz), 2.26(2 H on C-2,t,J = 7 Hz), 2.15(2 H on C-14, m), 2.03(2 H on C-17, quintet, J = 7 Hz), 1.6–1.4(4 H on C-3 and C-8,m), 1.30(8 H on C-4,C-5,C-6 and C-7,br s), 0.94(3 H on C-18,t,J = 7 Hz); ^{13}C NMR(CD_3OD): δ176.7(s), 135.8(d), 133.3(d), 129.4(d), 125.4(d), 75.0(2C,d), 72.4(d), 37.4(t), 34.2(t), 30.8(t), 29.5(2C,t). 29.3(t), 25.7(t), 25.2(t), 20.9(t), 13.9(q).

Using a similar extraction procedure a 270 g sample of freeze-dried deep-water *L. majuscula* collected at Sand Island pinnacle, Enewetak in February, 1976 yielded 0.4 g of **1**. The same amount of **1** was also isolated from a shallow-water variety collected at Kahala Beach, Oahu.

Acetylation of methyl malyngate. A suspension of methyl malyngate (24 mg), obtained by treatment of **1** with ethereal diazomethane, and 77 mg NaOAc in 2 ml Ac_2O was heated at reflux for 75 min and then allowed to stand at room temp. overnight. The mixture was evaporated *in vacuo* and the residue was extracted with $CHCl_3$ to give 38 mg of a brown oily solid. Gel filtration of this material on Sephadex LH-20 with $CHCl_3$–MeOH (1:1) gave 24 mg of methyl malyngate triacetate as an oil; ^1H NMR($CDCl_3$) δ5.65(2 H,m), 5.43(1 H,m), 5.30(3 H, overlapping m's), 5.00(1 H,m), 3.62(3 H,s), 2.32(2 H,t), 2.20–1.90(4 H, overlapping m's), 2.01(6 H,s), 1.99(3 H,s), 1.7–1.4(4 H,br m), 1.25(8 H,br s), 0.91(3 H,t,J = 7.5 Hz); MS(rel intensity) *m/e*439(<1,M + 2–OCH_3), 425(<1), 368(1), 366(<1), 348(3,M–2HOAc), 320(2), 317(1), 308(7), 307(9), 306(31,m–2HOAc–CH_2CO), 297(4), 290(3), 289(4), 288(6,M–3HOAc), 277(10), 275(9), 268(9), 239(10), 237(14), 227(18), 226(100,M–OAc–CH_2CO–$CHOAcCH_2CH{=}CHCH_2CH_3$), 208(20), 194(58), 193(12), 185(11), 149(36); high resolution MS *m/e* 439.2648(calcd for $C_{24}H_{39}O_7$,439.2696), 348.2296(calcd for $C_{21}H_{32}O_4$,348.2301), 306.2191(calcd for $C_{19}H_{20}O_3$,306.2195), 226.1576(calcd for $C_{13}H_{22}O_3$,226.1569).

Acetonalization of **1**. A soln of 63 mg of **1** and 1.5 mg of *p*-TsOH in 2 ml of 2,2-dimethoxypropane was allowed to stand at room temp. for 3.5 hr. The mixture was evaporated *in vacuo* and the residual oil was passed through a column of Sephadex LH-20 with $CHCl_3$–MeOH (1:1) to give 56 mg of **3** as a colorless oil; IR(neat) 3420, 1715, 968, 720 cm^{-1}; ^1H NMR(C_6D_6) δ6.58(2 OH,br s), 5.79(2 H on C-10 and C-11,m), 5.52(2 H on C-15 and C-16,m), 4.48(1 H on C-9 or C-12,m), 4.12(2 H on C-13 and C-9 or C-12,m), 2.20(6 H on C-2,C-14 and C-17,m), 1.58(3 H,*cis* Me on acetonide ring,s), 1.37(3 H,*trans* Me on acetonide ring,s), 1.7–1.1(12 H,br m), 0.95(3 H on C-18,t,J = 7.5 Hz); high resolution Ms *m/e* 367.2424(calcd for $C_{21}H_{35}O_5$,367.2485,M-1), 353.2332(calcd for $C_{20}H_{33}O_5$,353.2328,M–CH_3).

Periodate oxidation of **1**. To a soln of 65 mg of **1** in 3 ml MeOH was added dropwise a soln of 45 mg of periodic acid in 2 ml H_2O. The mixture was stirred at room temp. for 3 hr, the MeOH was removed *in vacuo*, the residue was distributed between H_2O and CH_2Cl_2 (6 × 5 ml), and the combined CH_2Cl_2 extract was dried and evaporated to give 41 mg of an oily white solid. Recrystallization from CCl_4 gave 19 mg of **2**, m.p. 74.5–77.5°, $[\alpha]_D$ + 25°(*c*1.0,$CHCl_3$); ^1H NMR($CDCl_3$) δ9.57(1 H on C-12,d,J = 8 Hz), 6.82(1 H on C-10,dd,J = 16,4.5 Hz), 5.8–4.7(2 OH,v br), 6.28(1 H on C-11,ddd,J = 16,8,1.5 Hz), 4.42(1 H on C-9,brtdd,J = 5.5,4.5 and 1.5 Hz), 2.33(2 H on C-2,br t), 1.45–1.75(4 H on C-3 and C-8,br m), 1.30(8 H on C-4,C-5,C-6 and C-7,br s); high resolution MS *m/e* 229.0853(calcd for $C_{12}H_{21}O_4$,229.1440,M + 1), 210.1257(calcd for $C_{12}H_{18}O_3$,210.1256,M–H_2O).

9(S),12(R),13(S)-*Trihydroxystearic acid* (**4**). A suspension of 132 mg of **1** and 50 mg of 10% Pd/C in EtOAc was stirred under H_2 for 3 hr. The catalyst was removed by filtration and the solvent was evaporated to give crude crystalline tetrahydromalyngic acid. Recrystallization gave a polymorphic white solid, m.p. 105–108.5°(acetone), 108.5–112°(MeOH-acetone), 112.5–115.5°($CHCl_3$), 114.5–122.5°(EtOH); $[\alpha]_D^{24}$ – 5.8°(MeOH,c 1.2); ^1H NMR(acetone-d_6) δ4.80–3.20(4 OH,br), 4.00(1 H,m), 3.45(2 H,m), 2.23(2 H,t,J = 7 Hz), 1.90–1.10(24 H,br), 0.88(3 H, br t); ^{13}C NMR(MeOH-d_4) δ 176.9(C=O), 75.1(2 CH–OH), 71.6(CH–OH), 37.7(CH_2), 34.4(CH_2), 33.9(CH_2), 32.9(CH_2), 32.4(CH_2), 30.0(CH_2), 29.7(CH_2), 29.5(CH_2), 28.9(CH_2), 26.0(2 CH_2), 25.4(CH_2), 23.0(CH_2), 13.8(CH_3); ^{13}C NMR(DMSO-d_6) δ174.3, 73.9, 73.7, 69.8, 37.3, 33.7(2C), 32.5, 31.7, 29.2, 28.8(2C), 28.7, 25.3(2C), 24.7, 22.3, 14.1; high resolution MS *m/e* 312.2298(calcd for $C_{18}H_{32}O_4$,312.2301), 223.1330(calcd for $C_{13}H_{19}O_3$,233.1334), 197.1539(calcd for $C_{12}H_{21}O_2$,197.1542), 171.1392(calcd for $C_{10}H_{19}O_2$,171.1385), 171.1022(calcd for $C_9H_{15}O_3$,171.1021).

Methyl tetrahydromalyngate, obtained by treating the acid with ethereal diazomethane, was compared directly with the methyl esters of the two erythro 9(*S*),12,13-trihydroxystearic acids (α and β isomers[5]) by tlc on 10% sodium arsenite silica gel with $CHCl_3$–MeOH(65:1):[9] methyl tetrahydromalyngate and the methyl ester of the α-acid both had R_f 0.33 whereas the methyl ester of the β-acid had R_f 0.52. Tlc comparison with the methyl esters of the two threo 9(*S*),12,13-trehydroxystearic acids (γ and δ isomers[5]) was not made; both, however, are reported to have higher R_f values than that of the α-acid methyl ester. The sample of α-acid had the following properties: $[\alpha]_D^{30}$ – 3.3°(MeOH,*c*0.9), contaminated with a small amount (~10%) of the more dextrorotatory (Table 1) β-acid by tlc analysis; ^{13}C NMR(MeOH-d_4) identical to that of tetrahydromalyngic acid above.

Ozonolysis of **1**. A soln of 113 mg of malyngic acid in 5 ml EtOH was cooled to – 15° and treated with excess Oz for 1.5 hr. The soln was purged with N_2 and 160 mg of tetramethylammonium borohydride was added. The resulting mixture was heated to 60° for 30 min and then allowed to stand at room temp. overnight. Acetone was added to destroy excess hydride, the soln neutralized with dil HCl and evaproated to dryness, and the residue treated with MeOH and the mixture reevaporated (repeated 3 times) to remove methyl borate. A 162 mg portion of the remaining white solid (296 mg) was subjected to gel filtration on Sephadex G-15 with 0.2 M HOAc to give 18 mg of 2-deoxy-D-ribitol as a gummy solid, $[\alpha]_D^{24}$ – 15°(MeOH, *c* 1.5)[lit.$[\alpha]_D$ – 18°(MeOH,*c* 0.6) for 2-deoxy-D-ribitol[10] and $[\alpha]_D$ + 13.6°(EtOH,*c* 1.1), for 2-deoxy-D-xylitol[11]].

A mixture of 16 mg of the 2-deoxy-D-ribitol, 0.6 ml of benzoyl chloride, and 1 mg of 4-dimethylaminopyridine was allowed to stand at room temp. for 2 weeks. Excess reagent was evaporated *in vacuo* and the residual oily solid was passed through a column of Sephadex LH-20 with $CHCl_3$–MeOH(3:2) to give 32 mg of oily 2-deoxy-D-ribitol tetrabenzoate which crystallized from hexane-$CHCl_3$, m.p. 129–129.5°, $[\alpha]_D^{22}$ – 16°($CHCl_3$,*c* 3.1)[lit.[10] m.p. 129°, $[\alpha]_D$ – 14°($CHCl_3$,*c* 1)].

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