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PHYTOTOXINS. I. A 1-AMINODIMETHYLHEPTADECAPENTOL FROM *ALTERNARIA ALTERNATA* F. SP. *LYCOPERSICI*

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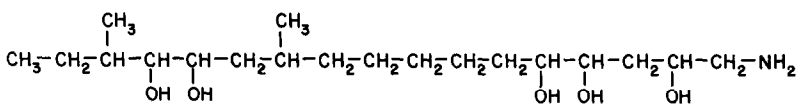
SUMMARY.- The products of alkaline hydrolysis of the host-specific phytotoxic fraction T_A from A. *alternata* f. sp. *lycopersici*[1] are identified as 1,2,3,-propanetricarboxylate and either 1-amino-11,15-dimethylheptadeca-2,4,5,13,14-pentol **1** or its 7,15-dimethyl isomer.

Host-specific phytotoxins, produced by more than 10 species of fungal pathogens, occur as diverse structural types and include relatively low-molecular weight cyclodepsipeptides,[2] terpenopeptides,[2] and unbranched long-chain polyketols.[3] Phytotoxins of this class are toxic only to the host that is susceptible to the pathogen which produces the toxin, and if they induce nearly all the symptoms of the disease are considered to be definitive chemical probes in the study of disease susceptibility and physiological stress at the molecular level.[2] In this and the accompanying paper[1] we describe evidence which shows that the ninhydrin-positive host-specific phytotoxic fraction T_A, produced in culture by the tomato-specific pathogen A. *alternata* f. sp. *lycopersici*,[4] consists of two esters of 1,2,3-propanetricarboxylic acid and the novel aminopentol **1**. The sites of esterification are a terminal carboxyl of the acid and C_{13} (major component **2a**) and C_{14} (**2b**) of **1**.

1



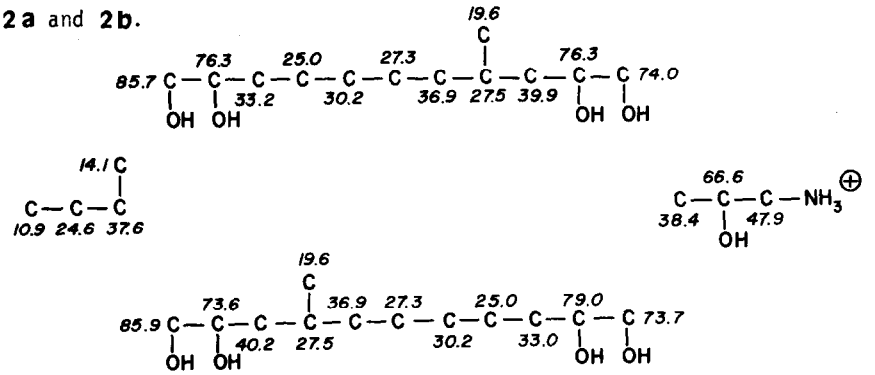
Treatment with 5 ml of boiling 5% aqueous NaOH for 10 min of 97.5 mg of the white, hygroscopic sodium form of T_A, $[\alpha]^{22}_{578}$ 22° (c 2.7, H_2O),[5a] gave 1,2,3-propanetricarboxylate[6] and the aminopentol **1**; **1** was extracted into butanol and isolated as the deliquescent hydrochloride **1a** (*ca.* 60 mg), $[\alpha]^{22}_{578}$ -15° (c 2.7, H_2O).[5b]

Methylation and deuteriomethylation[7] of **1** gave products whose HRMS[8] had base peaks of 360.3082 [$C_{20}H_{42}NO_4^+$ = 360.3113] and 378.4258 [$C_{20}H_{24}D_{18}NO_4^+$ = 378.4244] and parent peaks of 461.4041 [$C_{26}H_{55}NO_5^+$ = 461.4080; 0.41% BP] and 482.5347 [$C_{26}H_{34}D_{21}NO_5^+$ = 482.5398; 0.47% BP]. These data establish the molecular formula of **1** as $C_{19}H_{41}NO_5$ and show that it is a primary amine with 5 hydroxyl groups. The following are other noteworthy peaks in the HRMS of the deuteriomethylated product together with the molecular formulas established by comparison with the spectrum of the methylated product, the differences in ppm between the observed and calculated mass, and the ionizations relative to the base peak: 425.4665[M-C_4H_9; -6.8; 9.6%], 219.2522 [$C_5H_7(OCD_3)_3N(CD_3)_2^+$; -8.7; 1.06%], 172.2093 [$C_4H_6(OCD_3)_2N(CD_3)_2^+$; 1.4; 10.0%], 149.1644 [$C_5H_5(OCD_3)N(CD_3)_2^+$; 2.4; 3.8%], 137.1639 [$C_4H_5(OCD_3)N(CD_3)_2^+$; 7.2; 3.1%], 111.1482 [$C_2H_3(OCD_3)N(CD_3)_2^+$; -1.4; 18.0%], 110.1407 [$C_2H_2(OCD_3)N(CD_3)_2^+$; 1.3; 12.2%] and 104.1152 [$C_5H_{10}OCD_3^+$; -2.9; 70.3%].[9] The magnitude of the $C_5H_{10}OCD_3$ peak, together with the composition of the base peak and the presence of a significant M-C_4H_9 peak, indicate the formation of

$C_4H_9CHOCD_3CHOCD_3$ on deuteriomethylation of a $C_4H_9CHOHCHOH$ unit in **1**. Also, the composition of the nitrogen-containing fragments of < 219.3 daltons and the absence of a $C_3H_4(OCD_3)_2N(CD_3)_2^+$ peak strongly suggest the presence of the structural unit $CHXCHXCH_2CHXCHX$ in **1**, wherein X is OH or NH_2.

The CMR spectrum[10] of **1a** in D_2O-H_2O consists of 5 doublets at δ 64.94, 69.98, 70.49, 74.72 and 79.18 and a triplet at 45.28 ppm with J = 142-145 Hz, and two doublets at δ 28.69 and 36.05, 8 triplets at 23.96, 25.13, 25.72, 29.10, 31.72, 35.73 and 37.59, and 3 quartets at 10.47, 14.85 and 20.36 ppm with J = 122-126 Hz. (The proton-noise decoupled spectrum is shown in Figure 1A.) The chemical shift of the downfield triplet at δ 45.28 ppm agrees well with the value calculated[11] for an ammoniomethyl carbon (δ 47.9 ppm) with hydroxyls at C_2, C_4 and C_5, and not at all with that calculated for a hydroxymethyl carbon (δ 67.9-69.9 ppm) with two hydroxyls and an NH_3^+ at C_2, C_4 and C_5. Of the 3 possible structures for the C_4H_9 end of **1**, $(CH_3)_2CHCH_2CHOHCHOH$, n-$C_4H_9CHOHCHOH$ and $CH_3CH_2CH(CH_3)CHOHCHOH$, the first can be eliminated because it requires that at least two of the methyls have very similar chemical shifts (calc.[11] δ 22.1 ppm) whereas 3 well-separated methyl resonances are observed. The presence of two methine doublets in the CMR spectrum thus shows the presence of two CH_3CH units and leads to the conclusion that **1** is a 1-aminodimethylheptadecane with 5 hydroxyl groups.

What remains to complete the elucidation of the 2-D structure of **1** is to choose between the possible structures at the $C_4H_9CHOHCHOH$ end and to locate the one or two remaining methyls. The PMR spectrum of **1a** (see below) excludes C_6 as a site for a methyl group and requires that the C_4H_9 end be either $CH_3CH_2CH(CH_3)CHOHCHOHCH_2$ or n-$C_4H_9CHOHCHOHCH(CH_3)$. This leaves 9 possible structures, 5 with first unit and 4 with the other. Chemical shifts were calculated[11] for the 3 methyl and 2 methine carbons for these structures, and only those for the 7,15- and 11,15-dimethyl arrangement agree satisfactorily with those observed. Each complete set of calculated values for the two structures, given below, agrees well with the observed spectrum, and the final choice of the 11,15-dimethyl structure was made on the basis of the CMR spectrum of T_A, *i.e.*, **2a** and **2b**.



The 360-MHz PMR spectrum of **1a** in D_2O,[10] shown in Figure 1B, consists of 7 multiplets (1H each) from δ 4.063 to 2.938 ppm due to the 5 protons on carbinol carbons and the two diastereotopic ammoniomethyl protons, incompletely resolved bands from 650-350 Hz (18H), and, in the methyl region, two doublets at δ 0.932, J = 6.7 Hz, and 0.844 ppm, J = 6.8 Hz, and a triplet at δ 0.880 ppm, J = 7.5 Hz (9H). Consistent with the HRMS and CMR data, selective

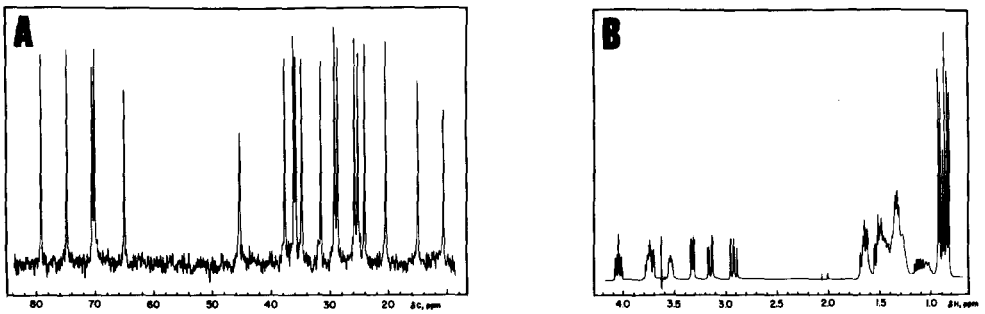
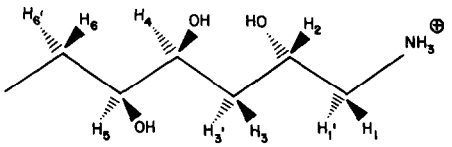


Figure 1: A, 50.3-MHz proton-noise decoupled CMR spectrum of **1a**; B, 360-MHz PMR spectrum of **1a**, resolution enhanced.

decoupling experiments established that the 7 downfield multiplets are due to 3 mutually coupled sets of protons, and that two upfield protons with chemical shifts of δ 1.533 and 1.650 ppm are both coupled with one proton of the 3-proton set (*i.e.*, protons at C_1 and C_2) and one proton of a 2-proton set. The chemical shifts at δ 3.340 and 3.776 ppm, J = 3.8 Hz, due to the isolated set of protons are assigned to protons at C_{14} and C_{13} because the first band is coupled with one upfield proton and the second is coupled with two.

The parameters of protons at C_1-C_5, which follow, are the same or nearly the same as those seen in the spectrum of T_A: δ_1 3.164, $\delta_{1'}$ 2.938, δ_2 4.063, δ_3 1.533, $\delta_{3'}$ 1.650, δ_4 3.739, δ_5 3.556, δ_6 *ca.* 1.43, $\delta_{6'}$ *ca.* 1.43 ppm; $J_{1,1'}$ = -13.1, $J_{1,2}$ = 3.0, $J_{1',2}$ = 9.9, $J_{2,3}$ = 3.0, $J_{2,3'}$ = 9.9, $J_{3,3'}$ = -14.8, $J_{3,4}$ = 10.8, $J_{3',4}$ = 2.0, $J_{4,5}$ = 4.5, $J_{5,6} \approx J_{5,6'}$ = 6-8 Hz. The vicinal coupling constants for protons along C_1 to C_5 are all significantly different from 6-8 Hz, and this indicates that there is a preferred conformation for this part of **1a**. Only two enantiomeric conformations are compatible with the observed coupling constants, and they require the all-**R** or all-**S** configuration.



We consider it suggestive that C_2, C_4 and C_5 of the all-**S** configuration shown have the same relative configuration as C_5, C_3 and C_2 of **D**-Glucose and C_5, C_3 and C_2 of 6-amino-4,6-dideoxy-**D**-*xylo*-hexose obtained from 4'-deoxykanamycin A.[14]

Results from the detached-leaf bioassay[4] indicated that the specific activity of the aminopentol derived from T_A is less than 5 x 10^{-5} that of T_A, the greatest differential tested.

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5a. Found: C, 55.22%; H, 8.59%; N, 2.66%. $C_{25}H_{46}NO_{19}Na$ requires: C, 55.24%; H, 8.53%; N, 2.58%.
b. Found: C, 53.90%; H, 9.98%; N, 3.31%. Calc. for 94.5% $C_{19}H_{42}ClNO_5$, 5.5% inert (NaCl?): C, 53.91%; H, 10.00%; N, 3.31%. Microanalyses were carried out at the Microanalytical Laboratory, University of California, Berkeley.
6. 360-MHz PMR spectrum[10] of the 1,2,3-propanetricarboxylic acid obtained: δ_1 3.147, δ_1' 3.207 δ_2 3.662 ppm; $J_{1,1'}$ = -17.1 Hz, $J_{1,2}$ = 6.0 Hz, $J_{1',2}$ = 7.7 Hz. C.J. Bastian, Jr., and R.B. Martin, *J. Phys. Chem.* **76**, 3073 (1972), reported, at 100 MHz: δ_1 3.139, δ_1' 3.213, δ_2 3.664 ppm; $J_{1,1'}$ = -17.2 Hz, $J_{1,2}$ = 5.8 Hz, $J_{1',2}$ = 7.6 Hz.
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8. Electron impact mass spectra were obtained with a modified AEIMS-902 using a quartz-tip probe, a repetitive scanning rate of 8 sec./decade (m/z 70-900) at 10000 resolution (10% valley definition), accel. voltage 8 kv, electron energy 60 ev, and source temp 250-260°; the data were processed using the Sigma 7-LOGOS-II real-time data acquisition computer. The high resolution mass spectra were obtained at the Biomedical and Environmental Mass Spectrometry Resource, Space Sciences Laboratory, University of California, Berkeley; the resource is supported by NIH Division of Research Resources grant RR 00719.
9. Other significant peaks: 481.5303 [M-H; -3.6; 0.86%], 464.4996 [M-CD_3; 4.4; 3.8%], 448.5018 [M-CD_3O; -1.8; 11.1%], 447.4961 [M-CD_3OH; 2.8; 8.4%], 429.4506 [M-C_2D_6OH; -4.4; 15.6%], 413.4591 [M-$C_2D_6O_2H$; 3.6; 10.3%], 412.4525 [M-$C_2D_6O_2H_2$; 6.7; 12.7%], 383.3926 [M-$C_4H_3D_9NO$; 3.1; 5.6%], 379.4296 [$C_{19}{}^{13}CH_{24}D_{18}NO_4^+$; 4.9; 34.6%], 362.3891 [$C_{18}{}^{13}CH_{25}D_{15}NO_4^+$; -11.6; 6.5%], 361.3849 [$C_{19}H_{25}D_{15}NO_4^+$; -13.8; 26.4%], 345.3926 [$C_{19}H_{25}D_{15}NO_3^+$; -6.8;
10. CMR spectra were obtained with an NT-200 spectrometer; chemical shifts are reported relative to external TMS (capillary) at δ 0.00 ppm and are precise to ± 0.02 ppm. PMR spectra were obtained at 200-MHz and 360-MHz with NT-200 and NT-360 spectrometers; chemical shifts are reported relative to internal acetate at δ 1.903 ppm and are precise to ± 0.002 ppm. The spectrometers were made available through the UCD NMR Facility; the NT-200 spectrometer was purchased in part by NSF grant CHE 79-04832 to the Department of Chemistry. Multiplicities of the bands in the CMR spectrum were determined by complete decoupling and off-resonance decoupling of the PMR spectrum; assignments to bands in both spectra are consistent with the results of the off-resonance decoupling experiments.
11. CMR chemical shifts were calculated using the "Lindeman-Adams Rule"[12,13] and the empirical substituent parameters compiled by Wehrli and Wirthlin.[13] Values calculated for carbons without correction for α, β or δ substituents are expected to have a standard error of 0.8 ppm;[13] values for substituent parameters "are reliable only within a margin of ± 5 ÷ 10%."
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