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A NEW SECALONIC ACID. LINKAGE BETWEEN TETRAHYDROXANTHONE UNITS DETERMINED FROM DEUTERIUM ISOTOPE ^{13}C CHEMICAL SHIFTS[1]

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Summary: The previously unknown secalonic acid G has been isolated from *Pyrenochaeta terrestris*, and its structure determined by circular dichroism and ^{13}C nmr techniques including deuterium isotope ^{13}C chemical shift measurements to identify the linkage between the tetrahydroxanthone units.

The structures of the enantiomeric 2,2'-linked homodimeric secalonic acids A (2) and D have been unequivocally established (1-4). Those of the other secalonic acids B, C, E (3) and F are also known (1, 5, 6), except for the linkage between tetrahydroxanthone units which is assumed to be the same as in 2. In these compounds the methyl and carbomethoxy substituents have a fixed *trans*-orientation and only the relative orientation of the C-5 hydroxyl group changes (Figure). During recent studies on the biosynthesis of this type of metabolite in five strains of *P. terrestris* (7) we isolated a new member of the series, secalonic acid G (1), in addition to 2 and 3. The structure of 1 has been elucidated by a combination of ^{13}C nmr and circular dichroism studies, and a general method of determining the linkage between tetrahydroxanthone units in such compounds has been developed using deuterium isotope ^{13}C chemical shift techniques. A recent report (8) that derivatives of secalonic acids may be potentially useful antitumor agents accounts for the renewed interest in this class of compounds.

The fungus was grown on a starch (5%)-neopeptone (1%) medium and one of the five strains tested, *P. terrestris* C-44-1A, produced superior yields of 1 (143 mg l^{-1}). Mycelium collected by filtration was leached with acetone and the aqueous phase left on removing the acetone was extracted with ethyl acetate. The residue from the extract was chromatographed on a silicic acid column from which 1 was eluted with a 4:1 benzene-ethyl acetate mixture. Crystallization from ethanol gave 1 as yellow needles, m.p. 206-207°; $[\alpha]_D^{20}$ -106.9° (c, 1.055, $CHCl_3$), -201.6° (c 1.105, C_5H_5N); $M^{+\cdot}$ 638.1614 (calcd. for $C_{32}H_{30}O_{14}$, 638.1635); $\lambda_{max}^{dioxane}$ 240, 265, and 338 nm (log ε 4.34, 4.28 and 4.57); ν_{max}^{KBr} 3600 (free OH), 3550 and 3450 (hydrogen-bonded OH), 1735

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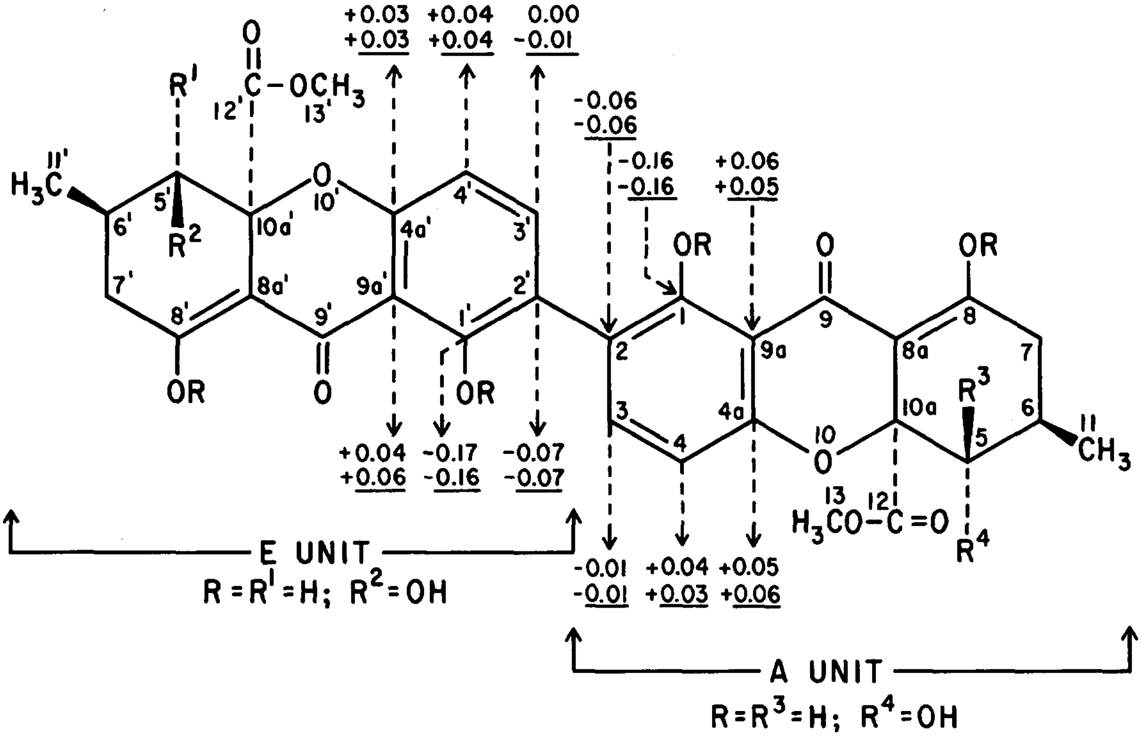

1; $R=R^1=R^3=H$; $R^2=R^4=OH$

2; $R=R^2=R^3=H$; $R^1=R^4=OH$

3; $R=R^1=R^4=H$; $R^2=R^3=OH$

$1a$; $R=R^1=R^3=H$; $R^2=R^4=\mathrm{O\overset{\overset{O}{\|}}{C}H}$

$2a$; $R=R^2=R^3=H$; $R^1=R^4=\mathrm{O\overset{\overset{O}{\|}}{C}H}$

$3a$; $R=R^1=R^4=H$; $R^2=R^3=\mathrm{O\overset{\overset{O}{\|}}{C}H}$

Figure. Deuterium isotope ^{13}C chemical shifts [$\Delta\delta_c = \delta_{C(O^2H)} - \delta_{C(OH)}$ppm] for the aromatic carbons of the diformate derivative of secalonic acid G (1a) in C^2HCl_3. Values for corresponding carbons in the same derivative of secalonic acids A (2a) and E (3a) are underlined in the A and E units.

(aliphatic C = O) and 1600 cm^{-1} (hydrogen-bonded C = O). The uv and ir spectra are similar to those of other secalonic acids (1, 4-6).

The ^1H nmr spectrum of 1 in pyridine is a composite of two different sets of resonances arising from A (or D)- (1,4) and E (or B)- type (1,5) tetrahydroxanthone units, and so are the ^1H-broadband decoupled and high resolution ^{13}C nmr spectra which will be reported elsewhere. A comparison of the circular dichroisim curve for 1 with those for 2 and 3 and their enantiomers permits the absolute configuration at C-5 (C-5'), C-6 (C-6') and C-10a (C-10a') to be deduced. Thus a large negative minimum at 331-333 nm in the curve of 1 dissolved in dioxane indicates an *S*-configuration at both C-10a and C-10a' as in the case of 2 and 3 (9). In addition the molar circular dichroism ($\Delta\varepsilon$, deg cm^2 decimole^{-1}) of 1 ($\Delta\varepsilon$ -15.8) falls between the values obtained for 2 ($\Delta\varepsilon$ -18.9) and 3 ($\Delta\varepsilon$ -13.8) under the same conditions and contrasts with the positive values given by secalonic acids B (1) and D (4). This evidence establishes that 1 is a heterodimer containing an A and an E unit, but additional information is required to identify the linkage between the units.

In principle the presence of adjacent hydrogens on the aromatic rings of secalonic acids limits the linkage between the tetrahydroxanthone units to the 2,2'-, 2,4'- or 4,4'-positions. Possibilities for 1 are further restricted by the nmr evidence which requires the linkage position to be at C-2 of the A unit as in 2 and to be the same as in 3 at the E unit. The precise location of the linkage at the E unit cannot be identified because the type of linkage in 3 was not established by previous work (5). Chemical shift substituent effects and spin-spin coupling considerations (10) also indicate that the ^{13}C nmr spectral features for the aromatic carbons of secalonic acids are likely to be relatively insensitive to the linkage position and a change of configuration at C-5. The linkage in 1 (and 3) was therefore determined from ^{13}C chemical shift changes which are induced when hydroxyl hydrogens are replaced by deuterium (11) and which can be used to correlate the hydroxyl group at C-1 (C-1') with the presence (2-linkage) or absence (4-linkage) of a substituent at C-2 (C-2') (eg 12, 13).

Compounds 1, 2 and 3 rearrange when left in pyridine solution for extended periods so they were converted to derivatives 1a, 2a and 3a which are soluble and stable in CHCl$_3$. When the hydroxyl hydrogens in 2a are replaced by deuterium a characteristic upfield deuterium isotope ^{13}C chemical shift (11) permits aromatic carbons bearing hydroxyls (C-1, C-1') to be distinguished from those bearing oxygen (C-4a, C-4a') (Figure). ^{13}C nmr analysis of 2 enriched with [1,2-^{13}C]acetate in a recent biosynthetic study (7) has shown that C-1 (C-1') and C-2 (C-2') are introduced as an intact ^{13}C-^{13}C unit. This unequivocally assigns the ^{13}C resonances for C-1 (C-1') and C-2 (C-2'). It is noteworthy that C-2 (C-2') also experiences an upfield deuterium isotope ^{13}C chemical shift whereas the other aromatic carbons are deshielded or not affected to the same extent. As the magnitude and direction of the deuterium isotope ^{13}C chemical shifts for the corresponding aromatic carbons of 1a and 3a agree with those for 2a the monomeric units in 1a, and also 3a, must be 2,2'-linked. Thus the combined evidence requires secalonic acid G to have structure 1 in which the tetrahydroxanthone units correspond to those in 2 and 3.

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