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THE STRUCTURE OF A NEW TYPE OF PLANT GROWTH INHIBITOR EXTRACTED FROM IMMATURE TOBACCO LEAVES

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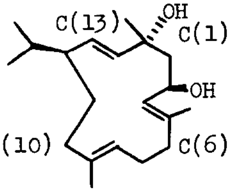
The development of axillary shoots, following the removal of upper leaves and inflorescence, represents a substantial problem in the cultivation of flue-cured tobacco.[2] Recently we indicated that the young upper-most leaves might control the growth of lateral shoots by producing natural growth inhibitors. We now wish to report that the structure of the most active of these inhibitors found so far is 4,8,13-duvatriene-1,3-diol (1).



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Isolation procedures for the active substances from Hicks variety tobacco have been given previously.[3] On silica gel chromatography (ethyl acetate/benzene, 55/45) there were two fractions with activity; the more polar was coded C2 and the less active, less polar compound, C1.

The crystalline material C2 (mp 123°C) lost water easily in the mass spectrometer and gave a highest observable m/e of 288.2453 (calcd for $C_{20}H_{32}O$, 288.2453). The infrared spectrum clearly showed a broad hydroxyl absorption at

3400 cm^{-1}, gem-dimethyl absorptions at 1380, 1375 cm^{-1} and a trans double bond absorption at 980 cm^{-1}. The uv absorption had a λ_{max}^{EtOH} 208 nm (ϵ 8400) band indicating only isolated double bonds.

Crystals of C2 (1) formed in the trigonal space group $P3_121$ with a=16.980(2) and c=11.83(1) from either methylene chloride-heptane or ether saturated with water solutions. A complete set of data within a 2θ sphere of 114° was collected with an automated four-circle diffractometer using CuK_α radiation (1.5418Å) and an ω-scan technique. Of the 1502 unique reflections measured, 1182 were judged observed after correction for Lorentz, polarization and background effects ($I \geq 3\sigma(I)$). A multi-solution weighted tangent formula approach[4] was used to obtain an initial set of phases. The subsequent electron density synthesis revealed all nonhydrogen atoms.[5] Full-matrix least-squares refinements of positional and anisotropic thermal parameters reduced the standard crystallographic residual to its current minimum of 0.109. No hydrogen atoms have been included at this stage.

The gross structure of the plant growth regulator is identical to that of 4,8,13-duvatriene-1,3-diol previously described by Roberts and Rowland[6] without stereochemical detail. The physical properties of C2 are similar but not quite identical[7] to those of β-4,8,13-duvatriene-1,3-diol and those of C1 resemble α-4,8,13-duvatriene-1,3-diol.

The proton nmr spectrum[8] of 1 while similar to that reported by Roberts and Rowland for their β-isomer, shows differences in the positions of the olefinic proton resonances and the resonance of the proton on C(3).[9] The proton decoupled natural abundance carbon-13 spectrum of 1 shows six olefinic carbons and fourteen saturated carbons. The assignment of this spectrum is given in Table 1 and is based on off resonance decoupling experiments, comparison with the spectrum of the acetate derivative of this compound, and from the chemical shifts and substituent effects derived from known compounds.[10] The proton nmr spectrum of the acetate derivative of 1 is essentially identical to that of 1 with the exception that the resonance of the proton on C(3) is shifted downfield to 5.77 ppm. The magnitude of this shift suggests that this proton occupies a pseudo equatorial position in solution.[11] The carbon-13 spectrum of the acetate derivative is given in Table 1 for comparison.

The proton nmr spectrum of compound C1 differs from that of the biologically more active compound C2 (1) in the olefinic region.[12] Again, this spectrum is similar to that reported by Roberts and Rowland for their α-isomer, although there are slight differences in the olefinic region. The carbon-13 spectrum of compound C1 is also given in Table 1. Both the proton and carbon-13 spectra of compound C1 suggest that it differs from C2 in the configurations at carbons 1 and 3 and is identical in most other respects. Slight differences in chemical shifts probably result from differences in the conformation of the two isomers.

Both C1 and C2 (1) give 100% inhibition at 10^{-3} M in the wheat coleoptile bioassay. At 10^{-4} M both give 60% inhibition while at 10^{-5} M C2 gives 15% inhibition and C1 shows no activity. For comparison abscisic acid gives 73% inhibition at 10^{-5} M.

To our knowledge this is the first report of a member of the cembrene family demonstrating plant growth regulating properties.[13]

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TABLE 1

Carbon-13 Chemical Shifts[a]

Carbon/Compound	C2	Acetate C2[b]	C1
1	71.50	70.962	72.52
2	52.77	50.96	52.33
3	64.38	68.57	66.33
4	131.48	127.19	127.83
5	136.27	139.04	136.36
6	[38.87][c]	[38.92]	[38.92]
7	27.75	27.65	28.04
8	124.46	124.22	124.51
9	132.94	133.09	133.29
10	[36.53][c]	[36.63]	[36.92]
11	23.16	23.16	23.41
12	46.28	46.23	46.53
13	130.26	130.17	130.76
14	136.27	136.36	137.63
15	28.77	28.38	30.14
16	15.89	16.04	16.09
17	15.02	15.26	15.02
18	32.96	33.11	33.11
19	[20.53][c]	[20.48]	[20.68]
20	[19.41][c]	[19.36]	[19.41]

[a] In ppm downfield from TMS.

[b] $CH_3\overset{O}{C}$, $\delta = 170.69$ and 21.46 ppm.

[c] These assignments may be reversed.

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7. We have been unable to obtain authentic samples of either α or β-4,8,13-duvatriene-1,3-diol for comparison purposes.

8. Proton spectra were obtained on $CDCl_3$ solutions on a Varian HA-100 spectrometer operating in the field sweep mode with a probe temperature of 29°. Carbon-13 spectra were obtained on a JEOL PFT-100 spectrometer with the EC-100 data system. Spectra were recorded using an 8K transform, a spectral width of 5000 Hz, a pulse angle of 45° and a repetition rate of 3 sec. Compound 1 displayed the following distinguishable peaks: δ .81 (3H, J=6.5) and δ 0.85 (3H, J=6.5) for the two isopropyl methyl groups; δ 1.41 (3H) for methyl on C(1); two olefinic methyl groups at δ 1.53 (3H, broad) and δ 1.72 (3H, J=1.5) for the methyls attached to C(9) and C(5) respectively; δ 4.77 (1H, J=2.2, 8.1 and 10.0) for the proton on C(3); broad peaks at δ 5.02 and 5.32 for the two olefinic protons on C(4) and C(8); δ 5.19 (1H, J=15.2, 7.5) for the proton on C(13); and a sharp doublet at δ 5.44 (1H, J=15.2) for the proton on C(14).

9. Differences in concentration could account for small differences in our spectra and those reported by Roberts and Rowland.

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12. The following peaks are distinguishable: δ 0.82 (3H, J=6.5) and δ 0.85 (3H, J=6.5) for the isopropyl methyl protons; δ 1.37 (3H) for the methyl protons attached to C(1); δ 1.55 (3H, broad) and δ 1.74 (3H, J=1.0) for the two olefinic methyl groups on C(9) and C(5), respectively; δ 2.0 (3H, sharp) acetate methyl; δ 4.48 (1H, J=4.0, 7.0 and 9.5) for the proton attached to C(3); broad peaks at δ 5.09 and 5.31 for the olefinic protons attached to C(4) and C(8); a sharp doublet δ 5.32 (J=15.0) for the proton attached to C(14); and a quartet for the proton attached to C(13) at δ 5.45 (J=15.0 and 1.5).

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