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CYCLITOLS FROM *CROTON CELTIDIFOLIUS*

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Key Word Index—*Croton celtidifolius*, Euphorbiaceae, cyclitols, 1L-1-*O*-methyl-*myo*-inositol, *neo*-inositol, sitosterol

Abstract—*Croton celtidifolius* has been found to contain 1L-1-*O*-methyl-*myo*-inositol, *neo*-inositol and sitosterol Structure of 1L-1-*O*-methyl-*myo*-inositol pentaacetate, elaborated by NMR decoupling technique, is described

INTRODUCTION

In the Southern subtropical part of Brazil in Santa Catarina, there are 104 identified species of Euphorbiaceae belonging to 28 genera All 27 species of the genus *Croton* are used in folklore medicine, a few are grouped together and are known as 'árvore de sangue' Latex of many is known as a skin irritant In our continued search for new drugs from natural sources we became interested in *Croton celtidifolius* Baill, which grows in abundance in Santa Catarina, and isolated 1L-1-*O*-methyl-*myo*-inositol, *neo*-inositol and sitosterol from this source *Neo*-inositol, known as a synthetic compound [1], has also been isolated from soil [Angyal, S J, pers comm], but, to our knowledge, this is the first report of its occurrence in plants

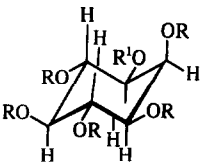
RESULTS AND DISCUSSION

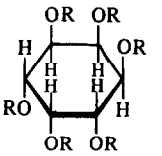
The compounds were isolated by solvent extraction, chromatography over acid washed alumina and subsequent purification of different fractions

The first compound, $C_{29}H_{50}O$ (M^+ 414), mp 139°, obtained from the chloroform extract, was identified as sitosterol by direct comparison with an authentic sample

Because of the lack of suitable equipment for the isolation of water soluble compounds, the highly polar fractions present in the methanol extract of the defatted plant were isolated and separated by acetylation of the crude mixture followed by chromatography Compound **1a**, $C_{17}H_{24}O_{11}$ (M^+ 414), mp 141°, $[\alpha]_D$ 12°, showed absorptions at 1750, 1430, 1360 and 1230 cm^{-1} in the IR for acetoxy and methoxy functions The electronic integration of all the resonance intensities in the ^1H NMR spectrum as well as the resonance patterns and positions of **1a** suggests that the four intense singlets around δ2 08 and the one at δ2 17 are for five acetoxy groups, the former four are *equatorial* and the latter *axial* [2] The other intense singlet at δ3 34 represents methoxy protons The multiplets in the region of δ4 88–5 78 and a pair of doublets at δ3 40 correspond to five methine protons attached to carbon atoms possessing the five acetoxy groups and one methine proton belonging to the carbon atom bearing the methoxy group respectively The spin decoupling experiments sort out the resonance patterns of the six methine protons (Fig 1) into three groups (i) three triplets at δ5 10, 5 38 and 5 49 corresponding to three *axial* protons of C-5, C-6 and C-4 and each of these resonances exhibit two 10 Hz splittings caused by spin–spin interactions of the vicinal *axial* protons, (ii) two pairs of doublets at δ3 40 and 4 97 representing two *axial* protons, each pair exhibiting 10 and 2 5 Hz couplings induced by *axial* and *equatorial* vicinal neighbours These two *axial* methine protons are to be at C-1 and C-3, (iii) a triplet at δ5 73 with two small splittings of 2 5 Hz corresponding to an *equatorial* proton located at C-2 The chemical shifts of the respective protons can very easily be accomodated by the stereostructure **1a**, identified as (+)-L-bornesitol pentaacetate

Compound **1a** upon hydrolysis with dry ammoniacal methanol at low temperature gave **1b**, $C_7H_{14}O_6$ (M^+ 194), mp 203°, $[\alpha]_D$ 32°, identified as 1L-1-*O*-methyl-*myo*-inositol Demethylation of **1b** with 57% aq hydriodic acid at reflux temperature [3] gave **1c**, $C_6H_{12}O_6$ (M^+ 180), mp 218–219°, established as *myo*-inositol Acetylation of **1c**, with acetic anhyride and dry pyridine by heating on a water bath gave **1d**, $C_{18}H_{24}O_{12}$ (M^+ 432), mp 216°, identified as *myo*-inositol hexaacetate In the NMR spectrum of **1d**, there were five intense singlets around δ2 00 for five *equatorial* acetoxy groups of C-1, C-3, C-4, C-5 and C-6 and a singlet at δ2 18 for the axial





1a R = Ac, R¹ = Me
1b R = H, R¹ = Me
1c R = R¹ = H
1d R = R¹ = Ac

R
2a Ac
2b H

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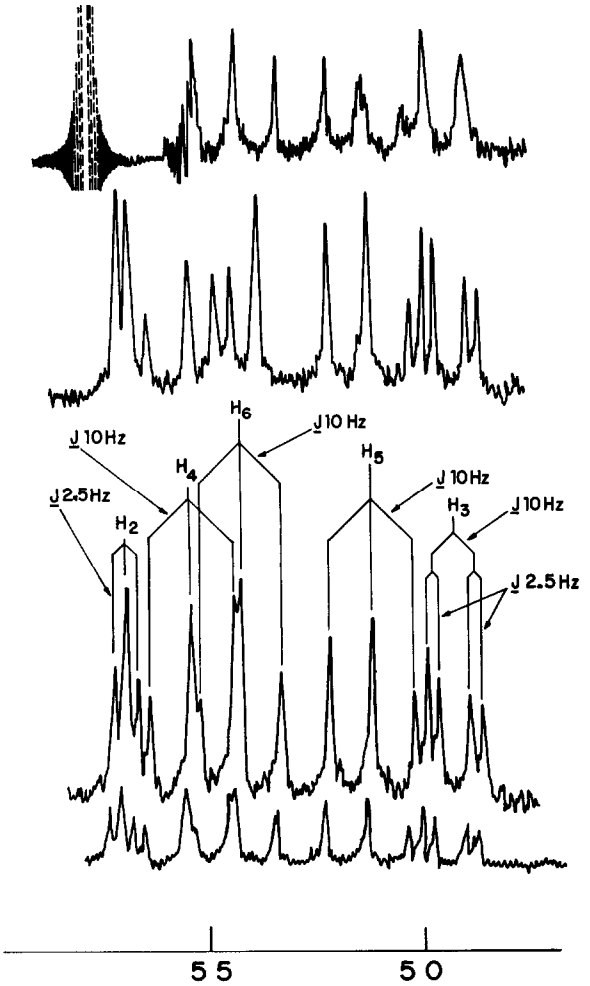

Fig 1 Spin decoupled 100 MHz ^{1}H NMR spectra of 1L-1-*O*-methyl-*myo*-inositol pentaacetate (**1a**)

acetoxy at C-2 In the methine region, the spectrum became more complex because of the downfield shift of the C-1 *axial* methine proton Otherwise the spectrum was almost the same as of **1a** excepting there was no methoxy singlet at δ3 34 and no pair of doublets at 3 40

Compound **2a**, $C_{18}H_{24}O_{12}$ (M^+ 432), mp 252–253°, ν_{max} 1760 and 1225 cm^{-1} showed an identical mass spectrum to **1d** In the methine region, the NMR spectrum of **2a** was almost identical with the NMR spectrum of **1d**, however, because of the paucity of material it was not possible to make a complete NMR study of **2a** to sort out each of the six methine protons and establish their stereoconfigurations Finally the compound was identified as *neo*-inositol hexaacetate by direct comparison with an authentic sample, mp, mmp and IR

Though both 1L-1-*O*-methyl-*myo*-inositol (**1b**, R = H, R′ = Me) and *neo*-inositol (**2b**, R = H) were isolated as pentaacetate (**1a**, R = Ac, R′ = Me) and hexaacetate (**2a**, R = Ac) respectively they both occur in *C celtidifolius* as the free cyclitols

EXPERIMENTAL

Mps are uncorr IR spectra were recorded as KBr discs and ^{1}H NMR spectra in $CDCl_3$ and C_6D_6 High resolution mass spectra were measured at 70 eV Non aq solvents were routinely dried over anhydrous Na_2SO_4 before use and Merck acid washed alumina was used for chromatography The analytical data were supplied by Micro-Tech Laboratories Inc, Skokie, IL 60076, U S A

Isolation Air dried finely ground leaves and twigs (1 kg) of *C celtidifolius*, collected from Santa Catarina, were extracted with $CHCl_3$(9 1) for 24 hr in a Soxhlet The conc $CHCl_3$ extract (8 g) upon chromatography over alumina (350 g) gave some oily material in the earlier fractions that was discarded, the latter fractions yielded solids (0 1 g), crystallized from $CHCl_3$–MeOH as colourless needles, mp 139°, was identified as sitosterol by mp, mmp and IR comparison with an authentic sample

The defatted plant material was then extracted in a Soxhlet with 95% MeOH (10 1) for 24 hr The red brown extract upon filtration, concn and complete removal of solvent gave a gummy material (3 g, *A*) which in the IR showed a broad band at 3400 and sharp peaks at 1430, 1355 and 1070 cm^{-1} for OH and OMe Gum *A* was freely soluble in hot H_2O Because of the lack of suitable equipment for the isolation of water soluble products, the gum was acetylated and worked up

Acetylatin of gum A Gum *A* (3 g) obtained from the MeOH extract was acetylated with Ac_2O (15 ml) and dry pyridine (5 ml) by heating at 100° for 2 hr, cooled and the brown mass was left in ice water overnight The sticky solid (2 g) that had separated upon working up followed by chromatography over alumina (150 g) using solvents of increasing polarity yielded a solid in the petrol–C_6H_6 (1 1) eluate This solid (**1a**, 0 25 g) after two crystallizations from petrol–C_6H_6 furnished colourless needles, mp 141°, $[\alpha]_D$ 12° (*c* 1 0, Me_2CO), ν_{max} 1750, 1430, 1360 and 1230 cm^{-1}, M^+ 404, *m/z* 362 $[M-42]^+$, 345, 331, 320, 301 and others with the base peak at *m/z* 43 It was established as (+)-L-bornesitol pentaacetate by NMR studies and by further comparison with an authentic sample of (−)-L-bornesitol pentaacetate, IR, mp and mmp (Calc for $C_{17}H_{24}O_{11}$ C, 50 49, H, 5 94, Found C, 50 51, H, 6 03%)

The $CHCl_3$ eluted part upon drying gave a second solid (**2a**, 0 01 g), crystallized from $CHCl_3$–EtOH mixture also in colourless needles, mp 252–253°, ν_{max} 1760 and 1225 cm^{-1}, M^+ 432, *m/z* 390 $[M-42]^+$, 330, 270, 199 and others with the base peak at *m/z* 43 It was identified as *neo*-inositol hexaacetate by direct comparison with an authentic sample by mp, mmp and IR (Calc for $C_{18}H_{24}O_{12}$, C, 50 00, H, 5 55, Found C, 49 79, H, 5 45%)

Hydrolysis of **1a** Dry powdered **1a** (0 3 g) was dissolved in dry MeOH (40 ml) saturated with dry NH_3 gas and left overnight in the refrigerator The solvent was removed and the solid (0 14 g) crystallized from EtOH–H_2O as colourless needles (**1b**), mp 203°, $[\alpha]_D$ 32° (*c* 1 0, H_2O) (Calc for $C_7H_{14}O_6$ C, 43 30, H, 7 22, Found C, 43 27, H, 7 30%)

Demethylation of **1b** Dry **1b** (0 12 g) in 57% aq HI (2 ml) was refluxed for 1 hr, HI removed in vacuum, cooled and the solid (0 1 g) crystallized twice from aq EtOH when pure *myo*-inositol (**1c**) was obtained in colourless needles, mp 218–219° (Calc for $C_6H_{12}O_6$ C, 40 00; H, 6 67, Found C, 39 93, H, 6 48%)

Preparation of **1d** Dry *myo*-inositol (0 06 g) dissolved in Ac_2O (2 ml) and two drops of dry pyridine, was heated at 100° for 1 hr, poured over crushed ice and left overnight The solid was filtered, washed with H_2O, dried and twice crystallized from $CHCl_3$–EtOH when pure *myo*-inositol hexaacetate (**1d**) was obtained (0 12 g) in colourless needles, mp 216°, ν_{max} 1765 and 1235 cm^{-1}, M^+ 432, *m/z* 390 $[M-42]^+$, 330, 270, 199 and others

with the base peak at m/z 43 The ^{1}H NMR spectrum was identical with the spectrum of *neo*-inositol hexaacetate (**2a**) (Calc for $C_{18}H_{24}O_{12}$ C, 50 00, H, 5 55, Found C, 50 04, H, 5 48%)

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CHEMODIFFERENTIATION OF DIOSGENIN IN *DIOSCOREA COMPOSITA*

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Key Word Index—*Dioscorea composita*, Dioscoreaceae, chemodifferentiation, diosgenin, tissue culture

Abstract—Diosgenin was isolated from different parts of a three-year-old plant of *Dioscorea composita* The amounts (% on a dry wt basis) present were tubers, 3 6, vine internodes and nodes with their leaves from first 20 nodes from the tubers, 1 6, similarly from intermediate 20 nodes, 0 039 and from upper 20 nodes, 0 03 The amounts (% on a dry wt basis) from tissue culture of nodal explants were 30-day-old callus, 0 89, 90-day-old callus, 1 61, emergent shoots, 2 5, regenerated roots, 0 08

INTRODUCTION

Diosgenin is used as a starting material for the manufacture of steroid drugs, including corticosteroids and oral contraceptives It is present in different amounts in the same plant growing in different localities [1, 2], but little is known regarding its levels in different parts of the plant and its synthesis in relation to organogenesis in culture

RESULTS AND DISCUSSION

In three-year-old plants harvested prior to flowering the upper young 20 nodes with leaves contained 0 030% diosgenin on a dry weight basis, whereas the nodes (20) adjacent to the tubers contained 1 6%, the intermediate 20 nodes contained 0 039% and the tubers contained 3 6% diosgenin. The side shoots arising at the nodes were not included in these analyses Young callus (30-day-old) obtained from MS_1 medium contained 0 89% and the highest content, 1 61%, was observed in 90-day-old culture The content was further enhanced to 2 52% in the emergent shoots derived from callus cultures However, regenerated roots contained less diosgenin (0 08%)

Studies with tissue cultures of *D composita* have shown that supplementation of the growth medium with 0 5 mg benzyladenine stimulates diosgenin biosynthesis [3, 4], and it has been reported that diosgenin synthesis is greater in unorganised tissue culture than in organised root culture of *D deltoidea* [5, 6] In the present study, however, it was found that diosgenin synthesis is greatest in organised shoot cultures of *D composite*, a finding which is reflected in the *in situ* production pattern of the plant Perhaps, chemodifferentiation of diosgenin is influenced by organogenesis A similar response has been reported in the case of cardenolide biosynthesis in *Calotropis gigantea* [7]

EXPERIMENTAL

Plant materials (aerial part, tubers) were collected from the University garden Three-year-old plants were divided into four parts viz the internodes and leaves of the upper 20 nodes, similarly of the 20 nodes adjacent to the tuber and likewise the 20 intermediate nodes, and tubers Nodal explants of healthy growing plants were cultured in RT (revised tobacco medium), supplemented with 2,4-D (2 mg/l) and Kn (0 5 mg/l) for callus initiation and organogenesis Diosgenin was also determined from the initiated callus (1-month-old), old callus (90-days-old), the emergent shoots derived from callus, proliferated roots in culture and regenerated plants in culture

Extraction procedure of diosgenin Dried finely powdered plant material (0 5 kg in the case of tubers and aerial parts, 10 g in the