Tetrahedron Vol 47, No 40, pp 8515-8522, 1991
Printed in Great Britain

0040-4020/91 $3 00+ 00
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GAUDICHAUDIOSIDES A-E, FIVE NOVEL DITERPENE GLYCOSIDE CONSTITUENTS FROM THE SWEET-TASTING PLANT, *BACCHARIS GAUDICHAUDIANA*

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(*Received in USA 9 August* 1991)

ABSTRACT- A new potently sweet labdane diterpene arabinoside, gaudichaudioside A (**1**), was isolated from the aerial parts of *Baccharis gaudichaudiana* DC (Compositae) Also isolated were four other novel labdane arabinosides, gaudichaudiosides B-E (**2-5**), which although closely related to **1** structurally, were not found to be highly sweet

INTRODUCTION

As part of our continuing search for sweet compounds of plant origin[1], we have investigated *Baccharis gaudichaudiana* DC (Compositae), a plant known locally as "chilca melosa" and used traditionally as an antidiabetic remedy in Paraguay Field inquiries showed that the leaves and stems of this plant exhibited a discernibly sweet taste, accompanied by some bitterness Subsequent laboratory investigation of a sample of *B gaudichaudiana* aerial parts collected from its natural habitat revealed that the sweetness was found to concentrate into a 1-butanol-soluble extract Fractionation of the 1-butanol and ethyl acetate extracts obtained from this *B gaudichaudiana* sample has led to the isolation of five novel labdane-type diterpene glycosides, gaudichaudiosides A-E (**1-5**), which possess unusual differential taste characteristics Among the five compounds, gaudichaudioside A (**1**) was found to be highly sweet, and is the prototype member of a new class of intensely sweet substances

Lithium-ion catalyzed high-resolution fast-atom-bombardment mass spectrometry (HR-FABMS) suggested that the molecular formula of gaudichaudioside A (**1**) was $C_{25}H_{40}O_8$ Assignments of the ^{1}H- and ^{13}C-NMR spectra of **1** were made with the use of ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR and selective INEPT NMR experiments Analysis of ^{13}C-NMR spectra (APT and SFORD), showed that 3 methyl, 9 methylene, 6 methine, 2 quaternary, 4 double-bond and 1 carbonyl carbon signals were present in the molecule of **1** (Table 1) The presence of an arabinosyl moiety was

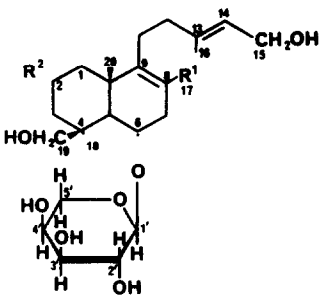
indicated that the latter substituent is in the α-equatorial position The configuration around the C-14,15 double bond was established as *E* (*trans*) from an NOE cross-peak between H_3-16 and the terminal allylic protons. Thus, the structure of gaudichaudioside A (1) was elucidated as 15,19-dihydroxylabda-8(9)-13(14)*E*-dien-17-al-6α-*O*-α-L-arabinopyranoside

Table 1. ^{13}C-NMR Assignments of Gaudichaudiosides A-E (1-5)[a]

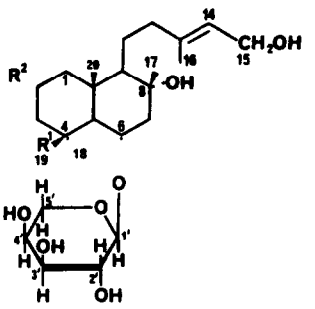
Carbon no.	1	2	3	4	5
1	37 1 (t)	38 8 (t)	47 8 (t)	40 6 (t)	50 1 (t)
2	19.0 (t)	19 6 (t)	64.9 (d)	18 0 (t)	64 8 (d)
3	38 9 (t)	40 2 (t)	47 1 (t)	43 9 (t)	53 1 (t)
4	44 7 (s)	43 2 (s)	44 5 (s)	40 4 (s)	36 5 (s)
5	56.9 (d)	57 9 (d)	56 9 (d)	61 3 (d)	61 1 (d)
6	80 1 (d)	81 5 (d)	80 8 (d)	81 8 (d)	80 6 (d)
7	34 7 (t)	40 1 (t)	40 1 (t)	53 3 (t)	54 2 (t)
8	131 3 (s)	129 5 (s)	129 3 (s)	74 0 (s)	74 3 (s)
9	170 1 (s)	144 1 (s)	143 8 (s)	61 8 (s)	61 8 (s)
10	39 9 (s)	39 9 (s)	41 5 (s)	39 6 (s)	42 2 (s)
11	26 0 (t)	27.3 (t)	27 3 (t)	25 3 (t)	25 2 (t)
12	43 8 (t)	42.7 (t)	42 5 (t)	41 1 (t)	43 8 (t)
13	138 7(s)	139 8 (s)	140 1 (s)	140 5 (s)	140 5 (s)
14	125 2 (d)	124 5 (d)	124 2 (d)	124 1 (d)	124 3 (d)
15	59 0 (t)	59 3 (t)	59 2 (t)	59 3 (t)	59 4 (t)
16	16 3 (q)	16 3 (q)	16 3 (q)	16 3 (q)	16 3 (q)
17	194 9 (d)	62 7 (t)	62 5 (t)	24 6 (q)	23 4 (q)
18	31 5 (q)	32 4 (q)	31 1 (q)	32 6 (q)	37 3 (q)
19	66 9 (t)	67 9 (t)	67 3 (t)	68 5 (t)	25 1 (q)
20	21 5 (q)	21 7 (q)	22 8 (q)	16 7 (q)	18 0 (q)
1′	106 0 (d)	106 4 (d)	106 2 (d)	106 0 (d)	106 0 (d)
2′	72 9 (d)	72 7 (d)	72 6 (d)	72 7 (d)	72 6 (d)
3′	74 4 (d)	74 9 (d)	74 6 (d)	74 8 (d)	74 8 (d)
4′	69 5 (d)	69 8 (d)	69 9 (d)	69 8 (d)	69 4 (d)
5′	66 9 (t)	67 1 (t)	67 0 (t)	67 1 (t)	66 4 (t)

[a]Chemical shifts in ppm downfield from TMS Solvent, CD_3OD + D_2O (three drops), s = singlet, d = doublet, t = triplet, q = quartet

Gaudichaudioside B (2) exhibited an elemental formula of $C_{25}H_{42}O_8$ as determined by sodium-ion catalyzed high-resolution FABMS Comparison of the ^{13}C-NMR spectra of 1 and 2 (Table 1) revealed only one structural difference between these compounds, in that C-17 (δ 62 7) was hydroxylated in 2 and the aldehyde signal of 1 was missing In its NOESY NMR spectrum, 2 displayed a cross-peak between H_3-20 and H-6 which demonstrated the relative β-axial orientation of these protons The coupling constant (10 Hz) observed between H-5 and H-6 indicated that H-5 was in the α-axial position, and an NOE cross-peak between H-5 and H_3-18 suggested that the latter methyl group was



	R¹	R²
(1)	CHO	H
(2)	CH_2OH	H
(3)	CH_2OH	OH



	R¹	R²
(4)	CH_2OH	H
(5)	CH_3	OH

indicated by the resonances at δ 106 0, 72 9, 74 4, 69 5 and 66 9, which are closely comparable to standard values reported in the literature[2-7] This observation was confirmed after identification of L-arabinose on hydrolysis of 1 with 0 1 N HCl However, attempts to characterize the aglycone of 1 after acid hydrolysis were unsuccessful due to the apparent lability of this moiety of the glycoside Further analysis of the ^{13}C-NMR chemical shifts and comparison with related diterpenoids[8] indicated 1 as belonging to the labdane class of diterpenes The functionalities of 1 were inferred from observations of ^{13}C NMR resonances at δ 66 9 and 59 0 (two primary alcohol groups), δ 194 9 (an aldehyde group), δ 31 5, 21 5 and 16 3 (three methyl groups) and δ 170 1 and 131 3, and δ 138 7 and 125 2 (two double bonds) The signal at δ 125 2 was assignable to a protonated double-bond carbon That this carbon is adjacent to a primary alcohol group was suggested by the coupling of H-14 (δ 5 41) to H_2-15 (δ 4 10) in the ^{1}H-^{1}H COSY spectrum of 1 The anomeric proton signal at δ 4 35 (d, J = 6 0 Hz) indicated the configuration of the L-arabinose unit to be α[4] The selective INEPT NMR technique[9] was used to decide the position of the saccharide unit attachment to the aglycone of 1 Irradiation of the anomeric proton NMR signal at δ 4 35 ($^3J_{CH}$ = 4 Hz) enhanced C-6 of the aglycone at δ 80 1 Analogous irradiation of H-5 at δ 1 59 ($^3J_{CH}$ = 4 Hz) enhanced C-6, as well as other signals for C-10 and C-20 (δ 39 9 and 21 5, respectively) The COLOC (Correlation spectroscopy via LOng range Couplings) NMR experiment[10] was utilized to support the ^{13}C NMR chemical shift assignments of the quaternary carbons in the aglycone portion of 1 (Figure 1) Thus, in the COLOC spectrum of 1, two-bond heteronuclear correlations between C-4 and H-5, C-8 and H-17, C-10 and H-5, C-10 and H_3-20, and C-13 and H_3-16, and three-bond correlations between C-9 and H_3-20, and C-14 and H_3-16, confirmed the ^{13}C-NMR assignments of the quaternary carbons, C-4, C-8, C-9, C-10 and C-13, respectively The relative stererochemistry of 1 at the C-4, C-5 and C-10 chiral centers was determined from a 2-D NOE (NOESY) NMR experiment An NOE interaction was observed between H_3-20 and H-6, thus indicating their β-diaxial relationship Accordingly, it may be inferred that the L-arabinosyl unit of 1 is substituted equatorially, which was substantiated by the large coupling constant (10 Hz) observed between H-5 and H-6 in the ^{1}H-NMR spectrum A further NOE cross-peak between H-5 and H_3-18

in the α-equatorial position The configuration at the 14,15 double bond was determined as *E* (*trans*), after the observation of a cross-peak between H_3-16 and H_2-15 in the NOESY NMR spectrum of 2. Comparison of ^{13}C-NMR chemical shift values of the sugar portions of the glycosides 1 and 2 indicated that an L-arabinosyl moiety was affixed at C-6 in gaudichaudioside B (2) The α-anomeric configuration of the sugar unit was evident from the coupling constant (7 Hz) of H-1′ and H-2′[4] Therefore, gaudichaudioside B was elucidated as 15,17,19-trihydroxylabda-8(9)-13(14)*E*-dien-6α-*O*-α-L-arabinopyranoside

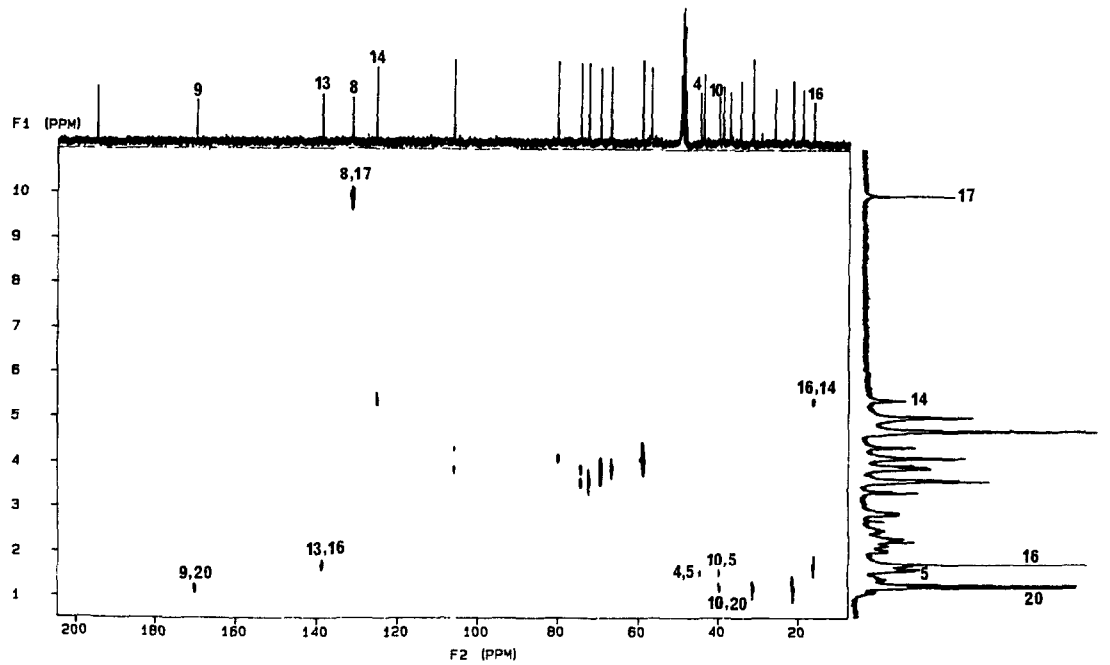


Figure 1. COLOC spectrum of gaudichaudioside A (1) in CD_3OD + D_2O (three drops) 128 scans were taken for each of 256 transients acquired over a period of 10 5 hr The spectrum was recorded on a Varian XL-300 spectrometer

Gaudichaudioside C (3) was found to have a molecular formula of $C_{25}H_{42}O_9$, as determined from high-resolution FABMS Comparison of the closely related ^{13}C-NMR data of glycosides 2 and 3 (Table 1) indicated that in the latter compound, a hydroxyl group occurred at position C-2 The deshielding of C-2, C-1 and C-3 by 45 3, 9 0 and 6 9 ppm, respectively, and the shielding of C-5 by 1 0 ppm are in close agreement with the corresponding values reported for equatorially substituted cyclohexanols[11] Further, the deshielding of C-4 and C-10 by 1 3 and 1 6 ppm, respectively, is in close accord with values reported for 2-equatorially substituted hydroxylabdane diterpenoids[12] The α-equatorial assignment of the 2-hydroxyl group in 3 was also supported by a NOESY NMR experiment Cross-peaks between H_3-20 and H-6, and H_3-20 and H-2 indicated that H_3-20, H-6 and H-2 were all in β-axial positions, and therefore

the 2-hydroxy group is situated α-equatorially The position of the sugar linkage was determined from ^{1}H-^{1}H COSY spectrum, wherein H-5 was found to be coupled to H-6 The sugar, its position of attachment and the anomeric configuration were determined in the same manner as for gaudichaudiosides A and B (1 and 2) It therefore follows that gaudichaudioside C (3) has the structure, 2α,5,17,19-tetrahydroxylabda-8(9)-13(14)*E*-dien-6α-*O*-α-L-arabinopyranoside.

Gaudichaudioside D (4), exhibited a molecular formula of $C_{25}H_{44}O_8$ using HR-FABMS Inferences from the ^{13}C-NMR spectral data of 4 (Table 1) indicated that, relative to gaudichaudioside A (1), the C-8, C-9 double bond and the C-7 aldehyde were absent, although the presence of tertiary hydroxy and tertiary methyl groups at C-8 in 4 were evident from the resonances at δ 74 0 and δ 24 6, respectively The configuration of the 8-hydroxy group was determined as α-equatorial from analysis of the chemical shifts of C-8, 9 and 17, as well as from NOE difference and 2-D NOE NMR experiments Thus, the ^{13}C-NMR chemical shifts of C-8, C-9 and C-17 (δ 74 0, 61 8 and 24 6, respectively) in 4 are in agreement with literature values reported for 8α-equatorially-substituted hydroxylabdanes, as compared to the corresponding chemical shift values of δ 74 0, 58 8 and 30 5 exhibited by 8-axially-substituted hydroxylabdanes[12-14] When H_3-17 was irradiated, an NOE enhancement was observed for H_3-20 and reciprocal irradiation of H_3-20 showed an NOE enhancement for the H_3-17 signal In a NOESY NMR experiment performed on 4, a cross-peak was observed between H_3-17 and H-6 It can thus be concluded that the hydroxy group at C-8 is α-equatorially oriented The β-equatorial position of the side chain at C-9 was inferred from the observation of an NOE cross-peak between H-5 and H-9 The structure of gaudichaudioside D (4) was therefore deduced as 8α,15,19-trihydroxylabda-13(14)*E*-en-6α-*O*-α-L-arabinopyranoside

The final glycoside we wish to report, gaudichaudioside E (5), was found to have the same molecular formula ($C_{25}H_{44}O_8$) as gaudichaudioside D (4) by HR-FABMS Comparison of its ^{13}C NMR data with those of 4 (Table 1) readily revealed that the C-4 hydroxymethyl group in 4 was missing and a secondary hydroxy group occurred at C-2 (δ 64 8) in 5 The α-equatorial orientation of the hydroxy group at C-2 was suggested by an NOE interaction between H-2 and H_3-20 in its NOESY spectrum Further NOEs between H-6 and H_3-20, H_3-17 and H_3-20, H_3-19 and H_3-20, and H_2-15 and H_3-16 allowed the assignments of the relative configurations as shown in the structure of 5 Thus, gaudichaudioside E (5) has the structure, 2α,8α,15-trihydroxylabda-13(14)*E*-en-6α-O-α-L-arabinopyranoside

Prior to being assessed for sweetness, the initial MeOH/H_2O, 1-BuOH and EtOAc extracts of the aerial parts of *B gaudichaudiana* and gaudichaudioside A (1) were shown to be non-toxic in preliminary acute toxicity tests in mice according to standard protocols[15-18] The MeOH/H_2O extract and compound 1 were also not mutagenic towards *Salmonella typhumurium* strain TM677 both in the presence and absence of a metabolic activating system[15-19] Gaudichaudioside A (1) was judged by a small taste panel as exhibiting about 55 times the sweetness potency of a 2% w/v aqueous sucrose solution Moreover, 1 was found to possess good hedonic properties, in being pleasant-tasting with a very low concomitant perception of bitterness Two other labdane derivatives are known to be highly

sweet, namely, the furanoditerpenes, baiyunoside[20,21] and phlomisoside-I[21] (+)-Baiyunol, the common aglycone of these sweet labdanes, and baiyunoside itself have recently served as target synthetic compounds[22,23] While baiyunoside exhibits a sweet taste that persists for more than 1 hr,[20] gaudichaudioside A (1) produced a rapid cut-off in its sweet effect Gaudichaudiosides B-E (2-5) were found to be sweet-bitter, neutral-tasting, wholly bitter and sweet-bitter, respectively, when tasted as 0 5% w/v aqueous solutions Although the sweet tastes of compounds 2 and 5 were not studied in detail by the test panel, these compounds produced an initial sweet sensation lasting a few seconds which was then replaced by an entirely bitter effect Compounds 1-5 therefore appear to exhibit a wider range of taste effects than has been noted before for any group of closely related natural products

EXPERIMENTAL

GENERAL PROCEDURES: The UV spectra were obtained on a Beckman DU-7 spectrometer and IR spectra were recorded on a Nicolet MX-1 FT-IR interferometer Optical rotations were measured with a Perkin-Elmer 241 polarimeter Melting points (uncorrected) were determined using a Kofler hot-stage instrument ^{1}H and ^{13}C NMR spectra were recorded with TMS as internal standard, employing either a Nicolet NT-360 or a Varian XL-300 instrument (360 MHz or 300 MHz, respectively) Low- and high-resolution mass spectra were obtained on a Finnigan MAT-90 instrument GC/MS analysis was performed on a Varian MAT 112S instrument

PLANT MATERIAL: The aerial parts of *B gaudichaudiana* were collected by three of us (D.D S , E B and A.D K.) near Pedro Juan Caballero, Amambay Province, Paraguay in August 1987 The plant was identified by D D S and a voucher specimen (Soejarto *et al* 6071) has been deposited in the John G Searle Herbarium, Field Museum of Natural History, Chicago, Illinois

ISOLATION OF COMPOUNDS 1-5: Above-ground parts of *B gaudichaudiana* (2 kg) were extracted with 80% methanol (3 x 10 l) by percolation over a total period of three days After removal of solvent *in vacuo*, the residue (380 g) was dissolved in 50% methanol in water (1 l) and partitioned successively with petroleum ether (3 x 1 l), ethyl acetate (3 x 1 l) and 1-butanol (3 x 1 l) The 1-butanol extract (65 g) was chromatographed over a Si gel column (650 g) using gradient elution, starting with $CHCl_3$ MeOH (95 5) with the polarity gradually increased to $CHCl_3$ MeOH (60 40) Fractions showing similar TLC profiles in $CHCl_3$ MeOH H_2O (6 3 1, lower layer), after spraying with 1% vanillin in sulfuric acid, were pooled Gaudichaudiosides A (1) and B (2) appeared in daylight as green and orange zones, respectively, after spraying with the reagent and heating at 110 ^{0}C for 10 min, while gaudichaudioside C (3) appeared as a pink zone The corresponding pooled fractions were then subjected to repeated flash chromatography over Si gel columns (100-150 g) to obtain pure gaudichaudioside A (1, 510 mg, 0 0255% w/w), gaudichaudioside B (2, 110 mg, 0 0055% w/w), and gaudichaudioside C (3, 65 mg, 0 0033% w/w) A portion of the ethyl acetate extract (60 g of 75 g) was applied over a Si gel column (800 g) and eluted with mixtures of $CHCl_3$ MeOH (9 1 to 1 1) The collected fractions were monitored by TLC in the same manner as for compounds 1-3 Those fractions showing similar TLC patterns were combined Gaudichaudiosides D (4) and E (5) appeared in daylight as pink zones after spraying with the reagent and heating at 110 ^{0}C for 10 min The resulting combined fractions, respectively were then subjected to repeated flash column chromatography over Si gel (300-400 g) to afford pure gaudichaudiosides D (4, 150 mg, 0 0075% w/w) and E (5, 160 mg, 0 0080%)

CHARACTERIZATION OF GAUDICHAUDIOSIDE A (1) Isolated as a white amorphous powder, mp 162-165^0, $[\alpha]_D$ +77^0 (MeOH, c 0 48), UV λ_{max} nm (MeOH) 225 (log ϵ 3 47), IR ν_{max} 3390-3327, 2910, 1664, 1625, 1095, 983 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD + 3 drops D_2O) δ 1 21 (3 H, s, H_3-20), 1 28 (3 H, s, H_3-18), 1 59 (1 H, d, J = 10 Hz, H-5), 1 73 (3 H, s, H_3-16), 2 78 (1 H, dd, J = 16, 10 Hz, H-7α), 3 56 - 3 63 (2 H, overlapping signals, H-5'a, 19a), 3 90 (1 H, m, H-4'), 3 93-3 95 (4 H, overlapping signals, H-2', 3', 5'b, 19b), 4 10 (2 H, d, J = 6 Hz, H_2-15), 4 19 (1 H, m, H-6), 4 35 (1 H, d, J = 6 Hz, H-1'), 5 41 (1 H, t, J = 6 Hz, H-14), 9 97 (1H, s, H-17), ^{13}C-NMR (90 8 MHz, CD_3OD + 3 drops D_2O, see Table 1), LR-FABMS (% rel int) 469 ({M + H}$^{+1}$, 46), 451(68), 319(45), 301(100), 203(52), 231(86), 158(46), HR-FABMS (M + Li)$^{+1}$, m/z 475 2884 for $C_{25}H_{40}O_8Li$, Δ +0 1 mmu

CHARACTERIZATION OF GAUDICHAUDIOSIDE B (2) White amorphous powder, mp 128-131°, $[\alpha]_D$ +60° (MeOH, c 0 1), UV λ_{max} nm (MeOH) 206 (log ϵ 3.59), IR ν_{max} 3200, 2725, 1670, 1375, 1037 cm^{-1}, ^1H NMR (300 MHz, CD_3OD + 3 drops D_2O) δ 1.11 (3 H, s, H_3-20), 1 27 (3 H, s, H_3-18), 1.56 (1 H, d, J = 10 Hz, H-5), 1 70 (3 H, s, H_3-16), 2 80 (1 H, dd, J = 17, 10 Hz, H-7α), 3.49-3.62 (2 H, overlapping signals, H-5'a, 19a), 3 72 (1 H, m, H-4'), 3 82 - 3 98 (4 H, overlapping signals, H-2', 3', 5'b, 19b), 4.10 (2 H, d, J = 7 Hz, H_2-15), 4 29 (1 H, d, J = 7 Hz, H-1'), 4 30 (1 H, m, H-6), 5 38 (1 H, t, J = 7 Hz, H-14), ^{13}C-NMR (90 8 MHz, CD_3OD + 3 drops D_2O, see Table 1), LR-FABMS (% rel int.) 471 ({M + H}$^{+1}$, 23), 303(100), 205(63), 145(46), 119(49), 109(47), HR-FABMS (M + Na)$^+$, *m/z* 493 2766 for $C_{25}H_{42}O_8Na$ = Δ-1 1 mmu

CHARACTERIZATION OF GAUDICHAUDIOSIDE C (3) White amorphous powder, mp 218-221°, $[\alpha]_D$ +66° (MeOH, c 0 1), UV λ_{max} nm (MeOH) 205 (log ϵ 3.66), IR ν_{max} 3200, 2725, 1670, 1375, 1037 cm^{-1}, ^1H-NMR (300 MHz, CD_3OD + 3 drops D_2O) δ 1 14 (3 H, s, H_3-20), 1 32 (3H, s, H_3-18), 1.58 (1 H, d, J = 11 Hz, H-5), 1.72 (3 H, s, H_3-16), 2.83 (1H, dd, J = 17, 5 Hz, H-7α), 3 57 - 3 62 (2 H, overlapping signals, H-5'a, 19 a), 3.86 (1H, m, H-4'), 3 91 - 3 96 (5 H, overlapping signals, H-2', 3', 5'b, 2, 19b), 4 10 (2 H, d, J = 6 Hz, H_2-15), 4 15 (1H, m, H-6), 4 34 (1H, d, J = 7 Hz, H-1'), 5 41 (1 H, t, J = 6 Hz, H-14), ^{13}C-NMR (90 8 MHz, D_2O + 3 drops D_2O, see Table 1), LR-FABMS (rel int) 487 ({M + H}$^{+1}$, 23), 461(31), 369(100), 333(24), 313(27), 301(33), HR-FABMS (M + H)$^+$, *m/z* 487 2900 for $C_{25}H_{43}O_9$, Δ -2.2 mmu

CHARACTERIZATION OF GAUDICHAUDIOSIDE D (4) White amorphous powder, mp 101-105°, $[\alpha]_D$ +49° (MeOH, c 0 10), UV λ_{max} nm (MeOH) 205 (log ϵ 3 78), IR ν_{max} 3375, 2925, 1375, 1350, 1125, 1060, 760 cm^{-1}, ^1H NMR (300 MHz, CD_3OD + 3 drops D_2O) δ 0 94 (3 H, s, H_3-20), 1 15 (3 H, s, H_3-17), 1 25 (3H, s, H_3-17), 1 25 (3 H, s, H_3-19), 1 36 (1 H, d, J = 10 Hz, H-5), 1 67 (3 H, s, H_3-16), 2 46 (1 H, dd, J = 9, 3 Hz, H-7α), 3 39 (1 H, d, J = 11 Hz, H-19a), 3 50 (1 H, dd, J = 10, 4 Hz, H-3'), 3 59 (1 H, br d, J = 12 Hz, H-4'), 3 91 (1 H, d, J = 11 Hz, H-19b), 4 02 (1 H, m, H-6), 4 06 (2 H, d, J = 7 Hz, H_2-15), 4 22 (1 H, d, J = 7 Hz, H-1'), 5 37 (1 H, t, J = 7 Hz, H-14), ^{13}C-NMR (90 8 MHz, CD_3OD + 3 drops D_2O, see Table 1), LR-FABMS (rel int) 471 ({M - H}$^{-1}$, 100), 469(14), 367(16), 275(81), 273(19), 205(28), HR-FABMS (M + H)$^{+1}$ *m/z* 473 3100 for $C_{25}H_{45}O_8$, Δ -1 4 mmu

CHARACTERIZATION OF GAUDICHAUDIOSIDE E (5) White amorphous powder, mp 103-106°, $[\alpha]_D$ +30° (MeOH, c 0 1), UV λ_{max} (MeOH) 205 (log ϵ 2 71), IR ν_{max} 3420, 2925, 1650, 110 cm^{-1}, ^1H-NMR (300 MHz, CD_3OD + 3 drops D_2O) δ 0 93 (3 H, s, H_3-20), 1 08 (3 H, s, H_3-17), 1 16 (3 H, s, H_3-19), 1.24 (1 H, d, J = 10 Hz, H-5), 1 3 (3H, s, H_3-18), 1 69 (3 H, s, H_3-16), 2 46 (1H, dd, J = 12 5, 3 4 Hz, H-7α), 3 50-3 60 (2 H, overlapping signals, H-2', 3'), 3 74 - 3 80 (3 H, overlapping signals, H-2, 4', 6), 4 07 (2 H, d, J = 7 Hz, H_2-15), 4 34 (1 H, d, J = 7 Hz, H-1'), 5.38 (1 H, t, J = 6 Hz, H-14), ^{13}C-NMR (90 8 MHz, CD_3OD + 3 drops D_2O, see Table 1), LR-FABMS (rel int) 471 ({M - H}$^{-1}$, 100), 469(29), 433(8), 415(19), 275(15), 243(10), HR-FABMS (M + H)$^+$ 473 3097 for $C_{25}H_{45}O_8$, Δ -1 7 mmu

HYDROLYSIS OF GAUDICHAUDIOSIDES A-E (1-5) A solution of each glycoside (5 mg dissolved in 0 5 ml MeOH and 0 5 ml 10% HCl) was stirred at 60-65°C for 3 hr[2]. Each mixture was extracted with chloroform and showed evidence of aglycone decomposition on TLC analysis No improvement in agycone stability resulted using less vigorous hydrolytic conditions When examined by TLC the chloroform extracts showed a number of unidentified zones indicating that the aglycones had decomposed Arabinose was identified in the aqueous layers by TLC comparison with a reference standard This observation was further confirmed by GC/MS analysis of the trimethyl silyl derivative of the sugar The aqueous solution was evaporated to dryness with a current of N_2 gas The residue was then heated with a few drops of SIGMA SIL-A® (Sigma Chemical Company, St. Louis, MO) at 70 °C for 20 min Excess reagent was removed with N_2 gas The resulting product was subjected to GC/MS analysis TMS-arabinose was identified by comparison with authentic standard arabinose treated in the same manner under the same GC conditions The optical rotation of the sugar obtained from the hydrolysis of 1-5, $[\alpha]_D$ +105° (90% MeOH, c 0 20) was compared with those of standard L-arabinose, $[\alpha]_D$ +111° (90% MeOH, c 0 20), and standard D-arabinose, $[\alpha]_D$ -108° (90% MeOH, c 0 20), which therefore established the presence of an L-arabinosyl substituent in gaudichaudiosides A-E (1-5)

SAFETY EVALUATION OF *B. GAUDICHAUDIANA* EXTRACTS AND GAUDICHAUDIOSIDE A (1): The initial 80% MeOH, EtOAc, and 1-BuOH extracts from *B gaudichaudiana* aerial parts and pure gaudichaudioside A (1) were evaluated for acute toxicity using male Swiss-Webster mice The test materials were administered by oral intubation at dose levels of 1 and 2 g/kg (extracts) and 1 g/kg body weight (pure 1) Procedures and protocols for

toxicological testing were followed as published previously[15-18] None of the samples tested caused any mortality, and body weights recorded on days 0 (prior to administration), 1, 3, 7, and 14 did not differ for treated versus control animals. The *B gaudichaudiana* 80% MeOH extract and pure gaudichaudioside A (1) were evaluated for mutagenicity at the dose ranges of 0 15-2 4 mg/ml and 0 08-1 36 mg/ml, respectively, and were found to be not mutagenic for *Salmonella typhimurium* TM677, both in the presence and absence of a metabolic activating system, when tested as described previously[15-19]

SENSORY EVALUATION OF GAUDICHAUDIOSIDE A (1): Gaudichaudioside A (1) was tested for its sweetness intensity relative to sucrose and evaluated for its sensory characteristics by a taste panel consisting of three persons[17-18] This diterpene arabinoside was rated as exhibiting 55 x the sweetness intensity of a 2% w/v aqueous solution of sucrose and was found to be pleasant tasting with very slight bitterness.

ACKNOWLEDGEMENT: This investigation was supported, in part, by a grant from General Foods Corporation, White Plains, New York, U.S.A. F F received fellowships from the World Health Organization (1986-1988) and from the Organization of African Unity (1988-1991) J M.P was the recipient of a Research Career Development Award from the National Cancer Institute, NIH (1984-1989). We are grateful to the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, and to Mr R Dvorak, College of Pharmacy, University of Illinois at Chicago, Chicago for the FAB mass spectral determinations The Research Resources Center, University of Illinois at Chicago, is gratefully acknowledged for the expert assistance and the provision of NMR facilities We also thank Dr K. Zaw of this institution, for helpful advice on the COLOC NMR experiment

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