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STERYL GLYCOSIDES AND ACYL STERYL GLYCOSIDES FROM *MUSA PARADISIACA**

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Key Word Index—*Musa paradisiaca*; Musaceae; sterols; sitosterol; stigmasterol; campesterol; 24-ethyllophenol; citrostadienol; steryl esters; steryl glycosides; sitosterol-β-D-glucoside; sitosterol 3-[β-D-glucopyranosyl(1″ → 6′)-β-D-glucopyranoside]; sitosterol 3-[*myo*-inosityl(1″ → 6′)-β-D-glucopyranoside]; acyl steryl glycosides; sitosterol 3-[6″-*O*-palmitoyl-β-D-glucopyranosyl(1″ → 6′)-β-D-glucopyranoside]; sitosterol 3-[2″-*O*-palmitoyl-*myo*-inosityl(1″ → 6′)-β-D-glucopyranoside]; seasonal variations; anti-ulcerogenic activity.

Abstract—From peeled fruits of *Musa paradisiaca* (banana, vegetable variety), two new acyl steryl glycosides, sitoindoside-III and sitoindoside-IV, and two new steryl glycosides, sitosterol gentiobioside and sitosterol *myo*-inosityl-β-D-glucoside, have been isolated by gradient solvent extraction and extensive chromatography (CC, prep. TLC, GC and HPLC). The compounds have been characterized by comprehensive spectroscopic analyses (IR, ^{1}H NMR, GC, mass spectra, $[\alpha]_D$) and crucial chemical transformation. Additionally, seasonal variations of the total sterols, free sterols, steryl esters, steryl glycosides and acyl steryl glycosides in the active samples of banana have been analysed. The results provide a basis for the observed fluctuations in the anti-ulcerogenic activity of the extracts, in different seasons, and the importance of appropriate formulation of the pure principles to optimize the activity.

INTRODUCTION

Fruits of *Musa paradisiaca* L. (banana, vegetable variety) have been used in the Indian system of medicine for the treatment of stomach ulcers for many years. In a previous paper, we reported the isolation, characterization and anti-ulcerogenic activity of two acyl steryl glycosides, viz. sitoindoside-I (**5**) and sitoindoside-II (**6**), from peeled fruits of an active variety of *M. paradisiaca* [1]. The additional findings that prompted this study were: (i) the anti-ulcerogenic activity was concentrated in the fruits of certain varieties of *M. paradisiaca* cultivated in the upper Gangetic plain; (ii) the activity progressively increased from August to October every year; and (iii) the two identified active constituents (**5** and **6**) accounted for only *ca* 33% of the activity of the total banana powder. The present paper reports the isolation, characterization and seasonal variations of the different types of sterol derivatives, e.g. total sterols (TS), free sterols (FS), steryl esters (SE), steryl glycosides (SG) and acyl steryl glycosides (ASG), from peeled fruits of the banana. The variations in the different chemical constituents have been analysed in the light of the observed variations in the anti-ulcerogenic activity of the total extracts in different seasons. Additionally, the importance of proper formulation of the pure principles to optimize the activity is apraised.

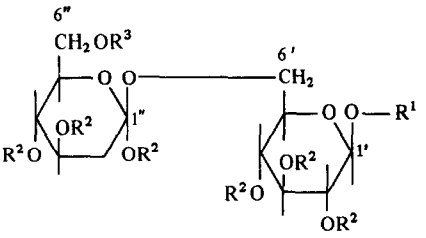
RESULTS AND DISCUSSION

Peeled fruits of *M. paradisiaca* L. (active vegetable variety), collected from June to August, fortnightly, showed on prep. TLC, HPLC, GC and mass spectral analysis different amounts of TS, FS, SE, SG and ASG. Extensive chromatography (CC, TLC, GC, HPLC) of the gradient solvent extracts afforded two new SGs, SG-I and SG-II, and two new ASGs, named sitoindoside-III and sitoindoside-IV, along with the two previously reported ASGs (**5** and **6**) [1], as the major entities. Additionally, the presence of several FSs, e.g. sitosterol, stigmasterol, campesterol, 24-ethyllophenol, citrostadienol, together with SE, composed of these sterols and the common fatty acids (linoleic, linolenic, lauric, myristic, palmitic and oleic/elaidic), and minor ASGs, composed of the same sterols, fatty acids, sugars (glucose, galactose, rhamnose) and *myo*-inositol, was detected by hydrolysis and spectroscopic analyses of the transformation products. Structure elucidation of the two new major ASGs only is described here.

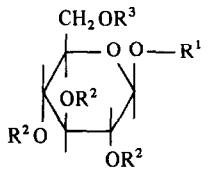
Sitoindoside-III

This compound, $C_{57}H_{100}O_{12}$ (by elemental analyses and $[M]^+$, FDMS), an amorphous solid, was optically active. It showed a light-purple colour with Liebermann–Burchard reagent, responded to the benzidine–metaperiodate test for polyols and exhibited only an end absorption in the UV spectrum. The IR and ^{1}H NMR spectra of the compound suggested the structural features of a fatty ester of a sterol glycoside. Deacylation of the compound with methanol–sodium methoxide gave a steryl glycoside (**3**) and methyl palmitate. The steryl glycoside, on further hydrolysis with emulsin [2], afforded sitosterol and D-glucose (identified as the alditol acetate). The permethyl ether [$C_{63}H_{112}O_{12}$, (**7**)] of sitoindoside-III, prepared according to ref. [3], on hydrolysis with methanol–hydrochloric acid gave palmitic acid, sitosterol and 2,3,4-tri-*O*-methylglucose (2:1 proportion with respect to the sterol). The methylated

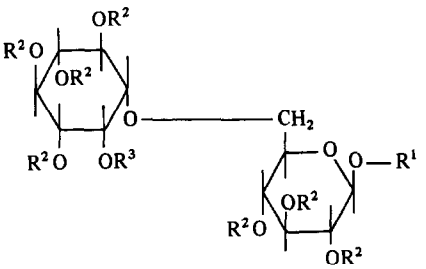
*Part 2 in the series "Bioactive Phytosterol Conjugates". For Part 1, see ref. [1].



1 R^1 = sitosteryl-3-, R^2 = H, R^3 = palmitoyl
3 R^1 = sitosteryl-3-, R^2 = R^3 = H
7 R^1 = sitosteryl-3-, R^2 = Me, R^3 = palmitoyl



5 R^1 = sitosteryl-3-, R^2 = H, R^3 = palmitoyl
6 R^1 = sitosteryl-3-, R^2 = H, R^2 = oleoyl



2 R^1 = sitosteryl-3-, R^2 = H, R^3 = palmitoyl
4 R^1 = sitosteryl-3-, R^2 = R^3 = H
8 R^1 = sitosteryl-3-, R^2 = Me, R^3 = palmitoyl

sugar was estimated as the alditol acetate by GC [4, 5]. Hence, the sitosterol 3-[6″-*O*-palmitoyl-β-D-glucopyranosyl (1″ → 6′)-β-D-glucopyranoside] structure (**1**) was assigned to sitoindoside-III. To our knowledge, this compound has not been encountered before in nature nor has it been prepared before synthetically.

Sitoindoside-IV

This compound, $C_{57}H_{100}O_{12}$, was optically active and exhibited spectral properties similar to those of **1**. It formed a crystalline acetate derivative, mp 146–148°, which in its ^1H NMR spectrum showed seven acetoxyl groups associated with sugar moieties. *O*-Deacylation of sitoindoside-IV, with methanol–sodium methoxide, gave methyl palmitate and a sitosterol glycoside (**4**). The latter on hydrolysis with methanol–hydrochloric acid gave 1 mol each of sitosterol, D-glucose and *myo*-inositol. The permethyl ether (**8**), of sitoindoside-IV, on acid hydrolysis afforded 1 mol each of sitosterol, palmitic acid, 2,3,4-tri-*O*-methylglucose and 3,4,5,6-tetra-*O*-methyl-*myo*-inositol. The *myo*-inositol derivative responded to the benzidine–metaperiodate test (indicating the presence of a vicinal dihydroxyl function) and also formed a ketal ($[M]^+$ at *m/z* 276) with acetone. Thus, sitoindoside-IV was assigned the sitosterol 3-[2″-*O*-palmitoyl-*myo*-inosityl(1″ → 6′)-β-D-glucopyranoside] structure (**2**). To our knowledge, sitoindoside-IV is the first example of a naturally occurring acyl steryl glycoside having a cyclitol moiety.

The identities of the two new SGs (SG-I and SG-II) were established as **3** and **4** by direct comparison with the *O*-deacylation products of **1** and **2**, respectively.

The TSs in banana fruits showed considerable seasonal variation (0.4–2.5%), the quantity being maximum in the samples collected in October. The proportions of ASGs and SGs in the TSs were also maximum (60–75%) during October. At other times (June–August), these are vicariously represented by the FS and SE. The relative abundance of the minor sterol conjugates, in October, was (in terms of the sterols) in the order: citrostadienol < 24-ethyllophenol < campesterol < stigmasterol < sitosterol. The fatty esters of 4,4-dimethyl- and 4α-methyltriterpenes were also found present as minor entities. The banana peel was reported to contain essentially the same FSs and triterpene constituents, although in considerably different amounts and relative proportions [6].

The anti-ulcerogenic activity of the ASGs (**1**, **2**, **5**, **6**) was determined, as such, and in combination with the respective SGs. Each of the four ASGs exhibited significant anti-ulcerogenic effects in the battery of tests accepted for this purpose [1]. Considerable synergism (30–70%) was also observed when the ASGs were administered in combination with the SGs. In order to elucidate the biochemical basis of anti-ulcerogenic activity, the membrane stabilizing action of the ASGs was evaluated. The total ASGs markedly potentiated the viability of certain cells [7]. The compounds also produced extensive proliferation [stimulation index (SI), 3.23 (on 48 hr exposure)] of splenic lymphocytes of mice (in doses of 5–10 μg/ml). The SI was comparable to that of a known mitogen, concanavalin A (Con A) (5 μg/ml, SI 3.20), administered under similar conditions. Thus, the ASGs should be regarded as potential immunostimulatory agents [8–10]. It would seem likely that the anti-ulcerogenic activity of the ASGs is mediated, at least in part, by a membrane stabilizing action, as was conjectured previously [1], and potentiation of viability of cells. Furthermore, ASGs and SGs are known to normalize arachidonic acid cascade events [K. H. Pegel, personal communication]. The bioactivity of the fruits of *M. paradisiaca*, therefore, resides in its acyl steryl glycosides.

As regards the observed difference in the anti-ulcerogenic activity of the pure ASGs (**5** and **6**) *vis-à-vis* the total banana powder or its methanol extract [1], the following rationale is cited. While SGs and ASGs, as they occur in plant materials, are readily and rapidly absorbed and assimilated by humans and animals, little medicinal effect is obtained when such pure substances are administered in the crystalline (or amorphous) form, orally or parenterally [11, 12]. The difference in regard to the absorption has been linked to their low solubility in water. In order to elicit optimal activity, a proper dosage form of these substances is necessary. Initial experiments, in our laboratory, with combined ASGs (**1**, **2**, **5**, **6**) dissolved in ethanol have exhibited promising results.

EXPERIMENTAL

General. Mps were determined on a Toshniwal apparatus and were uncorr. IR spectra were recorded on a Perkin–Elmer 257 instrument in Nujol or KBr pellets. 100 MHz ^{1}H NMR spectra were obtained on a Varian HA-100 spectrometer using TMS as an int. standard. MS were recorded on a MS-50 instrument. Silica gel/UV_{254} (Machery–Nagel) plates were used for TLC. Spots were detected by UV quenching and spraying with Liebermann–Burchard reagent (for sterols) and benzidine–metaperiodate reagent (for polyols). For GC analyses, the following columns and conditions were used. (i) [Diethyleneglycol succinate (10%) on 100–120 mesh, 3 m × 2 mm i.d., Gaschrom Q, operating temp. 165°, 40 ml/min N_2] for methyl esters of fatty acids; (ii) [Dexil (5%) on 100–120 mesh, 2 m × 2 mm i.d., Celite) operating temp. 260°, 40 ml/min N_2] for free sterols and steryl esters; and (iii) [Silar 10C (5%) on 100–200 mesh, 1.6 m × 2 mm i.d., Gaschrom Q, operating temp. 210°, 40 ml/min N_2] for cyclitol and alditol acetates. Prep. GC of sterols was performed on (6% OV-17 silicone on 60–80 mesh, Chromosorb Q, acid-washed, 5 m × 10 mm i.d.), operating temp. 280°, 250 ml/min N_2). Analytical and semi-prep. HPLC were carried out on a Waters Associates (model ALC 201) assembly fitted with a M-6000 pump, a U 6K injector, a C_{18} μ-Bondapak column (30 cm × 4 mm i.d., for analytical; 30 × 8 mm i.d., for semi-prep.).

Plant material. Musa paradisiaca L. (active, vegetable variety), was properly identified [1] and collected from the Varanasi district of Uttar Pradesh, from June to October, every week, for three consecutive years. The fruits were peeled, cut into small pieces and air-dried Different batches of the plant materials were preserved separately, in polythene bags, to prevent fungal infection.

Extraction. In a typical expt, dried and milled plant material (*ca* 1 kg), collected in the last week of October, was extracted × 3 (20 hr each) by stirring with MeOH at room temp. The combined MeOH extract were evaporated at 30°, to give a brown hygroscopic residue (22 g) (fraction A). This was then hot extracted (Soxhlet) with petrol (60–80°) and MeOH (30 hr each) in succession. The residues from the petrol and MeOH extracts were designated as fractions B (3 3 g) and C (2.1 g), respectively.

Treatment of fraction A. Half of this fraction (ca 11 g) was partitioned between Et_2O–H_2O (1:1) and the Et_2O extract was processed for neutral, phenolic and carboxylic fractions. The aq. mother liquor was extracted with EtOAc and the residue from the EtOAc extract was combined with fraction C. The neutral fraction (4.2 g) was chromatographed over silica gel (100 g) eluting with C_6H_6 (1 2 l.), C_6H_6–$CHCl_3$ (1:1, 1.5 l.), $CHCl_3$ (1.8 l.), $CHCl_3$–MeOH (99:1, 1 l., 95:5, 1.2 l.) and $CHCl_3$–MeOH–H_2O (90:8·2, 0.5 l.). Fractions (100 ml) were collected and monitored by analytical TLC. Four major fractions (a_1–a_4) were collected.

Fraction a_1. This fraction (1.2 g), obtained from the C_6H_6 and early C_6H_6–$CHCl_3$ eluates, was a mixture of mixed glycerides. On alkaline hydrolysis, it gave a mixture of fatty acids (listed in the decreasing order of concn)· palmitic, oleic/elaidic, linoleic, linolenic, lauric and myristic They were identified as the methyl esters by GC.

Fraction a_2. This fraction was obtained from the $CHCl_3$ eluates as a colourless solid (0.2 g), mp 124–145°, and was clearly a mixture. On TLC, it showed five Liebermann–Burchard positive spots for sterols Semi-prep. HPLC followed by GC, MS and ^{1}H NMR (where possible) established the composition of the mixture as sitosterol, mp 136°, $C_{29}H_{50}O$ ([M]$^+$, *m/z* 414); stigmasterol, mp 160–161°, $C_{29}H_{48}O$ ([M]$^+$, *m/z* 412); campesterol, mp 155–157°, $C_{28}H_{48}O$ ([M]$^+$, *m/z* 400); 24-ethyllophenol, mp 152–154°, $C_{30}H_{52}O$ ([M]$^+$, *m/z* 428), ^{1}H NMR ($CDCl_3$): δ5.2 (1H, *m*, H-7), 2.0–2.2 (methylenes), 0.7–1.0 (18H, Mes), 0.46 (3H, *s*, H-18), HPLC R_t 182 sec. (MeOH–$CHCl_3$–H_2O, 70:12:18); and 24-ethylidenelophenol (= citrostadienol), mp 161–162°, $C_{30}H_{50}O$ ([M]$^+$, *m/z* 426), ^{1}H NMR ($CDCl_3$): δ5.2 (2H, *m*, olefinic), 1.7 (3H, *d*, *J* = 6 Hz, H-29), 0.8–1.0 (15H, Mes), 0.48 (3H, *s*, H-18), HPLC R_t 220 sec.

Fraction a_3. This fraction was obtained from the later $CHCl_3$ and $CHCl_3$–MeOH eluates as a straw coloured hygroscopic solid (0.54 g). Analytical TLC suggested it to be a mixture of acyl steryl glycosides. A portion (0.14 g) was subjected to prep. TLC using $CHCl_3$–MeOH–HOAc–H_2O (90:8:1:1) as the developing solvent. Two major R_f regions *ca* 0.3 and *ca* 0.4 were scraped off and eluted with MeOH. The MeOH eluate from the R_f 0.3 region showed four major and several minor components on 2D TLC and analytical HPLC. The four major components were separated by semi-prep. HPLC using MeOH–$CHCl_3$–H_2O (70:12:18) and MeOH–H_2O (80:20) as eluants.

The first two components eluted were identified as sitoindoside-I (29 mg) and sitoindoside-II (7 mg) as previously [1].

Sitoindoside-III (**1**). This compound was obtained from the MeOH–H_2O eluates as an amorphous solid (32 mg); $[\alpha]_D^{20}$ −34.8° (MeOH); IR ν_{max}^{Nujol} cm^{-1}: 3400 (br, OH), 1730 (ester >CO), 1600 (br, sugar moiety); ^{1}H NMR (as the TMSi ether) ($CDCl_3$): δ5.3 (1H, *m*, H-6), 4.95 (1H, *d*, *J* = 7 Hz, glucosyl anomeric proton), 3.7–3.9 (13H, glucosyl protons), 1.2–1.35 (methylenes), 0.66–1.0 (21H, Mes); MS *m/z*: 976 [M]$^+$. (Found: C, 69.6; H, 11.8. $C_{57}H_{100}O_{12}$ requires: C, 70.0; H, 11.5.)

O-Deacylation of sitoindoside-III. Compound **1** (8 mg), dry MeOH (0.5 ml), and a freshly prepared soln (0.1 ml) of NaOMe (0.1 g Na dissolved in 100 ml MeOH) were mixed and stirred for 1 hr at room temp. The mixture was cooled, diluted with H_2O (2 ml) and Na^+ removed by treatment with Amberlite IR-120 (H^+). The aq. soln was processed as before [1] to give methyl palmitate (GC) and sitosterol gentiobioside, mp 268–270° (dec.); $[\alpha]_D$ −14.4° (C_5H_5N); ^{1}H NMR (TMSi ether): δ5.28 (1H, *m*, H-6), 4.9 (1H, *d*, *J* = 7 Hz, glucosyl anomeric H), 3.7–3.9 (13H), 0.68–1.0 (18H, Mes).

Enzymatic hydrolysis of sitosterol-3-O-gentiobioside. The above steryl glycoside (3 mg) was emulsified with emulsin (4 mg) in NaOAc–HOAc buffer (pH 5). The reaction mixture was worked-up in the usual way [2] to give sitosterol (GC) as the only aglucone. The glucone components in the aq. hydrolysate were converted to the alditol acetates [3, 5]. GC analysis showed the presence of only glucitol acetate (sitosterol–glucitol acetate, 1:2).

Acid hydrolysis of sitoindoside-III. This compound (11 mg) was dissolved in 2 M HCl (0.5 ml) and hydrolysed in a sealed tube at 100–105° (incubated) for 2 hr. The aldose contents in the aq. hydrolysate were estimated by GC of the corresponding alditol acetate(s) according to ref. [4]. Only glucitol acetate (2 mol/mol of sitosterol) was formed from the hydrolysis.

Permethylation of sitoindoside-III. To an ice-cold soln of this compound (14 mg), in Na-dried THF (5 ml), NaH (68 mg) was added and the mixture was stirred for 15 min. MeI (1 ml) was added and the stirring was continued for a further period of 30 min, under N_2. The solvent was evaporated, the residue redissolved in $CHCl_3$ and subjected to prep. TLC ($CHCl_3$–MeOH, 9:1). The R_f 0.25 zone was eluted with MeOH and worked-up in the usual way to provide the permethyl ether (**7**) as a syrupy material (11 mg), MS *m/z* (rel. int.): 1060 [M]$^+$ (4.5), 397 (25), 396 (100), 255 (40), 239 (5), 211 (11), 204 (18), 186 (7), 177 (7), 121 (8).

Hydrolysis of the permethyl ether. The permethyl ether (5 mg) was heated with MeOH–HCl (3%), in a sealed tube, at 100° for 30 min. The reaction mixture was cooled, diluted with H_2O and extracted with Et_2O. The Et_2O extract was worked-up to give

palmitic acid, from the carboxyl fraction (identified as the methyl ester by GC), and sitosterol (GC) from the neutral fraction. The aq. hydrolysate was worked-up as for the glucone components and estimated as before when 2 mol 2,3,4-tri-*O*-methylglucose per mol sitosterol was estimated.

Sitoindoside-IV (**2**) The more polar fraction from semi-prep. HPLC afforded compound **2** as a hygroscopic solid (27 mg), $[\alpha]_D$ −9.8° (MeOH); IR ν_{max}^{Nujol} cm^{-1}: 3400 (br), 1735, 1600, 1040, 835. (Found: C, 69.5; H, 11.2. $C_{57}H_{100}O_{12}$ requires: C, 70.0; H, 11.5.) The hepta-acetate, prepared with Ac_2O–Et_3N, crystallized from MeOH as off-white microcrystals, mp 146–148°; IR ν_{max}^{KBr} cm^{-1}: 1735, 1728, 1605, 1205; ^1H NMR ($CDCl_3$): δ5.3 (11H, *m*), 3.6–3.9 (3H, H-5′, H_2-6′), 2.0–2.1 (21H, OAc), 1.33–1.2 (methylenes), 0.68–1.0 (21H, Mes).

O-Deacylation of compound **2**, as before, gave methyl palmitate and the sitosteryl bioside (**4**). The latter, on hydrolysis with MeOH–HCl (3%) and usual work-up gave 1 mol each of sitosterol (GC, MS), *myo*-inositol (GC of the acetate, MS) and D-glucose (GC of glucitol acetate, MS).

The permethyl ether (**8**), prepared as before, was obtained as a glassy solid, MS *m/z* (rel. int.): 1074 $[M]^+$ (2). Acid hydrolysis afforded 3,4,5,6-tetra-*O*-methyl-*myo*-inositol (1 mol), 2,3,4-tri-*O*-methylglucose (1 mol) and sitosterol (1 mol). The monoacetonide of the tetra-*O*-methyl-*myo*-inositol (3 mg) was prepared by treating with Me_2CO (1 ml), in the presence of *p*-toluenesulphonic acid (5 mg). The mixture was stirred at room temp. for 2 hr. The reaction mixture was worked-up in the usual way and the product subjected to MS analysis, MS *m/z* (rel. int.): 276 $[M]^+$, (42), 246 (11), 233 (7), 58 (100).

Fraction a_4 afforded the residual ASGs. On saponification followed by acid hydrolysis the mixture showed the presence of sitosterol, stigmasterol, campesterol, 24-ethyllophenol, citrostadienol and cycloartenol (from the non-saponifiable fraction); glucose, galactose, rhamnose and *myo*-inositol; and C_{16}–C_{29} fatty acids (palmitic and oleic acids being the major entities).

Treatment of fraction B This fraction consisted essentially of a mixture of FS and SE The mixture was chromatographed on a column of Al_2O_3 (50 g, activity grade III), for partial purification, using increasing proportion of Et_2O in petrol (40–60°). The early fractions afforded an enriched mixture of SE (0.27 g from *ca* 0.6 g crude material). The later eluates gave a mixture of FS (0.24 g)

The mixture of SE (*ca* 0.2 g) was subjected to a sealed tube hydrolysis with MeOH–KOH (5%), at 110° for 1 hr. The reaction mixture was worked-up as for sterols and fatty acids. The sterols were identified as sitosterol, stigmasterol, campesterol and 24-ethyllophenol, as before The fatty acids were identified as (in decreasing order of abundance): palmitic, oleic/elaidic, lauric, myristic, linoleic and linolenic.

Treatment of fraction C. This fraction was chromatographed over silica gel (50 g, 22 × 2 cm) eluting with different proportions of $CHCl_3$–MeOH–H_2O. Five different fractions (c_1–c_5) were collected.

Fraction c_1. This fraction afforded sitosterol-β-D-glucoside (0.4 g), mp and mmp 283° (co-TLC, MS, HPLC); acid hydrolysis produced sitosterol and D-glucose.

Fraction c_2. This fraction afforded sitosterol gentiobioside (0 13 g), amorphous solid (co-TLC, $[\alpha]_D$, HPLC indistinguishable from **3**); acid hydrolysis gave sitosterol and D-glucose (2 mol/mol sitosterol).

Fraction c_3. This fraction was subjected to semi-prep HPLC, as before, when **4** (0.11 g) was obtained as a hygroscopic solid, $[\alpha]_D$ −21.4° (C_5H_5N); IR ν_{max}^{Nujol} cm^{-1}: 3350 (br), 1600 (br), 1040, 835; ^1H NMR (CD_3OD): δ5.3 (1H, *m*, H-6), 4 7 (1H, *d*, *J* = 7 Hz, glucosyl anomeric H), 3.9–4.4 (sugar H plus H_2O), 3.1–3.5 (carbinol methines), 1 9–2.1 (methylenes), 0 65–1.0 (18H, Mes). (Found: C, 64 6; H, 9 8. $C_{41}H_{70}O_{11}$ H_2O requires. C, 65 0; H, 9 5) On acid hydrolysis it gave sitosterol, D-glucose and *myo*-inositol (1:1:1).

Fraction c_4. This fraction (0.32 g), consisted of a mixture of strongly polar glycosides and was subjected to acid hydrolysis. The hydrolysed products were processed, as before, and analysed for the sterol and sugar components. There was no free sugar present in the parent mixture. The sterols were identified as sitosterol, stigmasterol, campesterol and a strongly polar sterol (*m/z* 446). The sugar components were glucose, rhamnose, galactose and *myo*-inositol plus three unidentified minor constituents.

Fraction c_5. This fraction contained essentially the same compounds as in fraction c_4 plus some metal ions (Cu^{2+}, Fe^{3+}, K^+). The metal ions were detected by atomic absorption spectroscopy [1].

Seasonal variations of FS, SE and ASG. The different batches of dried and milled banana powder were extracted with $CHCl_3$–MeOH (2.1) and MeOH, in succession, and the extracts were divided into small aliquots. The separation and quantitation of FS, SE and ASG in the different extracts were accomplished by CC, prep. TLC, GC and HPLC as described before.

Biological testing. ASGs were dissolved in H_2O. The soln was filtered through a 0.45 μm membrane and stored, in small aliquots, at 0°. Con A was dissolved in Pi buffered saline (PBS, 0.15 M), at a desired concn, membrane filtered and stored, in small aliquots, at 0°. The mitogenic activation (SI) of splenic lymphocytes of healthy adult male DBA/2 mice was determined as in ref. [9].

The viability of cells of an S-180 transplantable tumour of mice was tested with ASGs in the presence and absence of kalbretorine, an alkaloid of the Amaryllidaceae. Kalbretorine was found to cause marked reduction (from 75% down to 32.5%) in the viability of these cells [7]. The combined ASGs ($1–2 \times 10^{-2}$ M) annulled the effect of kalbretorine and further potentiated the viability of the cells (88%).

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