Pergamon

0031-9422(95)00549-8

Phytochemistry, Vol. 41, No. 2, pp. 599–603, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0031–9422/96 $15.00 + 0.00

OLIGOFUROSTANOSIDES AND OLIGOSPIROSTANOSIDES FROM ROOTS OF *ASPARAGUS FILICINUS*

S. C. SHARMA* and N. K. THAKUR

Department of Chemistry, Himachal Pradesh University, Summer-Hill, Shimla-171005, India

(*Received in revised form* 12 *June* 1995)

Key Word Index—*Asparagus filicinus*; Liliaceae; Steroidal saponins; Oligospirostanosides; Oligofurostanosides.

Abstract—The ethanolic extract roots of *Asparagus filicinus* contains a complex mixture of steroidal saponins, from which two oligospirostanosides (Filicinins A and B) and two oligofurostanosides (Filicinosides C and D) were characterized.

INTRODUCTION

In continuation of our studies on the chemical constituents of *Asparagus* spp. [1–8], *Asparagus filicinus* Buch-Ham. (Liliaceae), an indigenous plant of Himachal Pradesh and Punjab (India), well known for its medicinal properties [9] was selected for the present studies. We have previously reported [10] the isolation and characterization of furostanosides from the roots of this plant. A communication [11] with the characterization of different steroidal saponins from the roots of this plant has also recently been published.

RESULTS AND DISCUSSION

Filicinin-A (**1**) and Filicinin-B (**2**), two new oligospirostanosides obtained from an ethanolic extract of the roots of this plant were separated by column chromatography and crystallized from methanol. Their infrared spectrum showed well-defined spiroketal absorption bands [12–15], and these compounds showed positive to the Liebermann–Burchard test [16–17] and negative to the Ehrlich test [12, 18]. Acid hydrolysis [19–21] of **1** provided an aglycone sarsasapogenin and the neutralized aqueous hydrolysate contained D-glucose, D-xylose and D-galactose. Enzymatic hydrolysis [13, 22] of **1** with β-glucosidase revealed no β-D-glucose indicating that D-glucose is not the terminal sugar of the glycone moiety.

In order to find out the sequence of the sugars compound **1** was subjected to Kiliani hydrolysis [23]. Examination of the reaction mixture at intervals by paper chromatography showed that D-galactose and D-xylose appeared first must be the terminal sugars of the sugar chain. Two D-glucose molecules which appeared later are therefore the inner sugars through which D-xylose, D-galactose and D-xylose are linked to the aglycone sarsasapogenin (at C-3). The configurations of the sugars were deduced as β by Klyne's rule [24] and by ^{13}C NMR data.

Compound **1** was permethylated by modified Hakomori's method [13, 25] to yield a permethylate that which, no methanolysis followed by hydrolysis provided methylated sugars, identified by paper chromatography as 2,3,6-tri-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose and 2,3,4,6-tetra-*O*-methyl-D-galactose. These results clearly established that D-xylose and D-galactose are the terminal sugars linked through two molecules of D-galactose are the terminal sugars linked through two molecules of D-glucose with the aglycone at C-3.

In order to determine the exact linkages of the sugars with each other, compound **1** was subjected to partial hydrolysis [26–28] to produce four prosaponins PS_1 to PS_4. Acid hydrolysis of these prosaponins resulted in the same aglycone sarsasapogenin but different sugars, namely: D-glucose in PS_1 and PS_2; D-glucose and D-xylose in PS_3 and D-glucose and D-galactose in PS_4. Each prosaponin on permethylation followed by methanolysis and hydrolysis gave the following methylated sugars: PS_1, 2,3,4,6 tetra-*O*-methyl-D-glucose; PS_2, 2,3,6 tri-*O*-methyl-D-glucose and 2,3,4,6 tetra-*O*-methyl-D-glucose; PS_3, 2,3,6 tri-*O*-methyl-D-glucose, 2,3,4 tri-*O*-methyl-D-glucose, 2,3,4 tri-*O*-methyl-D-xylose; and PS_4, 2,3,6 tri-*O*-methyl-D-glucose (2 mol); 2,3,4,6 tetra-*O*-methyl-D-galactose. Hence, PS_1 = sarsasapogenin + glucose $(1 \rightarrow 4)$, $PS_2 = PS_1$ + glucose $(1 \rightarrow 4)$; $PS_3 = PS_2$ + xylose $(1 \rightarrow 6)$ and $PS_4 = PS_2$ + galactose $(1 \rightarrow 4)$. These results showed that the terminal sugars D-galactose $(1 \rightarrow 4)$ and D-xylose $(1 \rightarrow 6)$ are attached to a

*Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR chemical shifts of sugar moieties in D_2O of compound **1**

	Carbon Nos. Chemical shifts (ppm)					
Sugars	1	2	3	4	5	6
Glucose (*b*)	103.1	72.4	78.5	70.4	78.5	61.4
Glucose (*a*)	103.2	72.1	78.5	69.7	81.3	61.0
Galactose	103.9	71.0	73.1	69.0	75.2	61.3
Xylose	104.1	73.3	76.4	69.8	66.9	-

D-glucose (*a*) which, in turn, is linked with another glucose (*b*) through (1 → 4) linkage at C-3 of the aglycone.

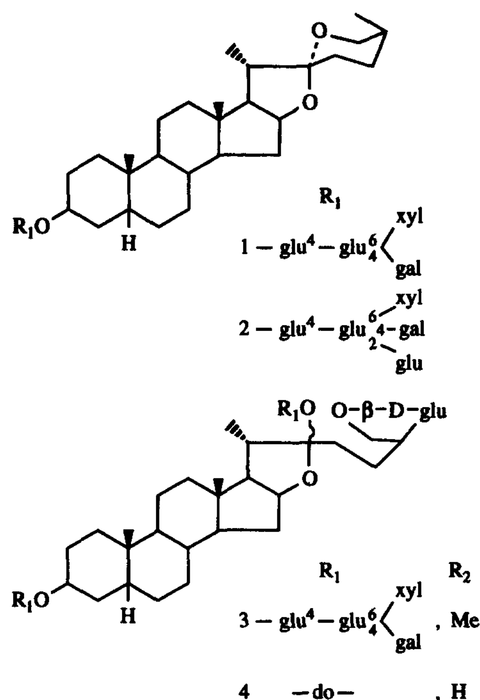
The FAB mass spectrum of **1** showed the molecular ion peak at *m/z* 1041 [M + Li]$^+$, indicating an aglycone of molecular weight 416 (sarsasapogenin), three hexose molecules (two glucose and a galactose) and one pentose. The ^{13}C NMR data of **1** (Table 1) also confirmed the presence of D-glucose and D-xylose as the terminal sugars because no downfield shift was observed for any of the carbon atoms, whereas in the D-glucose (*a*), there are downfield shifts for C-3′ and C-5′; for C-5′ this shift is greater than that for C-3, indicating linkages at C-4′ and C-6′. The D-glucose (*b*) also showed downfield shift for its C-3′ and C-5′ atoms, again showing the linkage at C-4′. Hence, the structure of filicinin-A(**1**) was established as 3-O-[{β-D-galactopyranosyl (1 → 4)} {β-D-xylopyranosyl (1 → 6)} β-D-glucopyranosyl (1 → 4)-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ol.

Enzymatic hydrolysis of **2** with β-glucosidase revealed D-glucose and prosaponin which was found identical to **1** (Co-TLC, superimposable infrared) indicating the presence of an additional terminal D-glucose in **2** than in **1**.

In order to determine the number of glucose molecules and their linkages with other sugar molecules, compound **2** was subjected to partial hydrolysis. It provided five prosaponins, PS_5 to PS_9, of which PS_5, PS_6, PS_7, and PS_9 were found to be identical to PS_1, PS_2, PS_3 and PS_4, respectively. PS_8, upon acid hydrolysis followed by the usual work up, showed only D-glucose on paper chromatography. Permethylation and methanolysis followed by hydrolysis of PS_8 provided three methylated sugars, namely, 2,3,6 tri-*O*-methyl-D-glucose, 3,4,6 tri-*O*-methyl-D-glucose and 2,3,4,6 tetra-*O*-D-glucose. These results indicated that in **2** the terminal glucose is attached with glucose (*a*) through (1 → 2) linkage along with D-galactose (1 → 4) and D-xylose (1 → 6) as in **1**.

Finally the FAB mass spectrum of **2** showed a molecular ion peak at *m/z* 1203 [M + Li]$^+$, indicating the presence of an aglycone of molecular weight 416 (sarsasapogenin), four hexose molecules (three glucose, one galactose) and one pentose (xylose).

From these data the structure for filicinin-B (**2**) was elucidated as: 3-O-[{β-D-glucopyranosyl (1 → 2)}{β-D-galactopyranosyl (1 → 4)} {β-D-xylopyranosyl (1 → 6)}-β-D-glucopyranosyl (1 → 4)-β-D-glucopyranosyl]-(25*S*)-5β-spirostan, 3β-ol.



Further column chromatography of the ethanol extract of *A. filicinus* roots yielded two oligofurostanosides, filicinoside-C (**3**) and filicinoside-D (**4**) which could not be separated by column chromatography. The infrared spectrum of this inseparable mixture of **3** and **4** exhibited no spiroketal absorption bands and gave a positive result with the Ehrlich test indicating its furostanolic nature. The mixture of **3** and **4**, on refluxing with dry methanol provided filicinoside C (**3**), while on refluxing with aqueous acetone yielded filicinoside-D (**4**). Both these compounds gave all the characteristic results in tests for oligofurostanosides [12–15, 18].

Enzymatic hydrolysis of the mixture of **3** and **4** with β-glucosidase liberated β-D-glucose and **1**, revealing glucose to be the terminal sugar. If a glucose molecule attached at C-26 of the oligofurostanoisde is liberated, the reaction mixture should become negative to the Ehrlich test with the closure of the F-ring and formation of the corresponding oligospirostanoside; this was found to be the case.

The question of whether D-glucose is not the terminal sugar of the main sugar chain attached with C-3 of sarsasapogenin was answered by the FAB-mass spectrometry. This FAB-mass spectrum of the mixture of **3** and **4** showed the molecular ion peak at *m/z* 1235 [M + Li]$^+$ indicating the presence of an aglycone of molecular weight 416 (sarsasapogenin), four hexoses and one pentose (open F-ring, 22-methoxyl). Therefore, it was clear that the additional D-glucose molecule exists as a terminal sugar linked with C-26 of the aglycone and automatically the other sugar chain at C-3 is the same as in **1**.

Table 2. ^{13}C NMR chemical shifts of sugar moieties in D_2O

	Carbon Nos. Chemical shifts (ppm)					
Sugars	1	2	3	4	5	6
Glucose (*b*)	103.2	72.5	78.4	70.4	78.4	61.3
Glucose (*a*)	103.1	72.1	78.4	69.7	81.3	61.0
Galactose	103.9	71.0	73.3	69.1	75.3	61.4
Xylose	104.1	73.2	76.5	69.8	66.0	–
Glucose (*c*)	103.4	73.3	75.3	70.4	75.3	61.3

In order to support further the above findings the mixture of **3** and **4** was subjected to partial hydrolysis to yield five prosaponins PS_{10} to PS_{14}. Of these prosaponins, PS_{12} tested positive to the Ehrlich test, indicating its furostanolic nature and was identical to filicinoside-B [10] (Co-TLC, IR), whereas the other prosaponins, PS_{10}, PS_{11}, PS_{13} and PS_{14}, were found to be identical to PS_1, PS_2, PS_3 and PS_4, respectively.

The ^{13}C NMR data of the mixture **3** and **4** (Table 2) confirmed all the above results, as all the sugars except a glucose (*c*) showed a similar pattern of shifts as in **1**. However, no downfield shift was observed for any carbon of glucose (*c*), thus proving its terminal position. Hence, these results established the structure of filicinoside-C as 3-O-[{β-D-galactopyranosyl (1 → 4)} {β-D-xylopyranosyl (1 → 6)}-β-D-glucopyranosyl (1 → 4)-β-D-glucopyranosyl]-26-O-β-D-glucopyransoyl-22-α-methoxyl-(25*S*)-5β-furostan, 3β, 26 diol and that of filicinoside-D as 3-O-[{β-D-galactopyranosyl (1 → 4)} {β-D-xylopyranosyl (1 → 6)}-β-D-glucopyransoyl (1 → 4)-β-D-glucopyranosyl]-26-O-β-D-glucopyranosyl-(25*S*)-5β-furostan-3β,22α, 26 triol.

EXPERIMENTAL

All mps were determined in open capillaries in an electrothermal mp apparatus at 2075 m above sea level and are uncorr. CC was carried out over silica gel (60–120 mesh, BDH) with $CHCl_3$–MeOH solvent system in order of increasing polarity. Homogeneity of the fractions was tested by TLC (silica gel G, BDH with binder) and spots were visualized by 8–10% H_2SO_4 and Ehrlich reagent followed by heating. PC (descending) was carried out on Whatman Filter paper No. 1 and spots were visualized by aniline hydrogen phthalate reagent. IR, EIMS, FAB-MS and ^{13}C NMR spectra were recorded on Perkin Elmer, Jeol D-300, Jeol SX-102/DA-6000 and Bruker WM-400, respectively. The solvent systems used were: A, $CHCl_3$–MeOH–H_2O (65:35:10); B, C_6H_6–EtAc (9:1) C, C_6H_6–petrol (1:1); D, *n*-BuOH–AcOH–H_2O (4:1:5) E, *n*-BuOH–EtOH–H_2O (5:1:4), F, $CHCl_3$–MeOH–H_2O (60:50:10).

The roots of *Asparagus filicinus* were collected from Hatwar, Distt. Bilaspur (HP), India.

Extraction and isolation. The roots were air dried, powdered (1 kg) and defatted with petrol (3 × 5 hr), EtAc (2 × 6 hr) and exhaustively extracted with EtOH (4 × 6 hr). The ethanolic extract was concd *in vacuo* and precipitated dropwise in large volumes of Me_2CO with constant shaking. The resulting residue was purified and separated by CC to yield a mixture of components out of which two oligospirostanosides, filicinin-A (**1**), filicinin-B (**2**) and two oligofurostanosides, filicinoside-C(**3**) and filicinoside-D(**4**) were separated and characterized.

Filicinin-A (**1**). Compound **1** was crystallized from MeOH. mp 187–9, $[\alpha]_D^{20}$ $-49°$ (MeOH), R_f 0.52 (Solvent-A, 3.7 g). It was positive to Liebermann–Burchard test and negative to Ehrlich test. IR ν_{max} cm^{-1}: 920 > 900, 255 spiroketal. FAB-MS *m/z*: 1041 $[M + Li]^+$. ^{13}C NMR: Table 1.

Acid hydrolysis. Acidic hydrolysis of **1** (150 mg) with 8–10% H_2SO_4 (50 ml) was carried out by refluxing on a steam bath for 4 hr. The usual work up provided an aglycone, crystallized from MeOH as needles. mp 194–6, $[\alpha]_D^{20}$ $-72.5°$ ($CHCl_3$) sarsasapogenin, Lit mp. 198–9°, $[\alpha]_D^{20}$ $-75°$ ($CHCl_3$), R_f 0.61 (solvent B). IR^{KBr} ν_{max} cm^{-1}: 3400–3350 (OH), 985, 920, 898, 860 [920 > 898 cm^{-1}, 25*S*]. EIMS *m/z*: 416$[M]^+$, 398, 357, 347, 344, 302, 287, 284, 273, 269, 255, 139 (base peak), 122 and 115. Its acetate was prepared in the cold in the usual manner and crystallized as needles from MeOH. mp 140–2°, $[\alpha]_D^{20}$ $-68.1°$ ($CHCl_3$) [sarsasapogenin acetate, Lit mp 143–4°, $[\alpha]_D^{20}$ -70 ($CHCl_3$), R_f 0.54 (solvent C). IR^{KBr} ν_{max} cm^{-1}: (OH) nil, 982, 920, 901, 860.

The aq. hydrolysate was neutralized with $BaCO_3$, filtered and conc. *in vacuo*. PC studies (solvent D) revealed the presence of D-galactose (R_f 0.16), D-glucose (R_f 0.18) and D-xylose (R_f 0.28).

Enzymic hydrolysis. Compound **1** (100 mg) was taken up in H_2O (25 ml) and β-glucosidase (10 mg) was added to along with toluene (3 drops) to cover the aq. layer. The reaction mixture was kept at room temp. for 72 hr. The PC (solvent D) did not show the presence of D-glucose whereas TLC (Solvent-A) showed only one spot corresponding to **1** (R_f 0.52) itself.

Kiliani hydrolysis. Compound **1** (100 mg) was treated with Kiliani mixture (50 ml, AcOH–H_2O–35% HCl, 35:55:10) at room temp. PC (solvent D) after 24 hr showed two spots corresponding to D-xylose (R_f 0.28) and D-galactose (R_f 0.16). PC after 48 hr and 72 hr showed one more spot corresponding to D-glucose (R_f 0.18) but its intensity doubled after 72 hr. There was no change on PC after 96 hr, even upon heating.

Permethylation. Compound **1** (300 mg) was permethylated by modified Hakomori's method (NaH, MeI, DMSO/N_2 atm) to get a permethylate (275 mg) which was purified by CC, R_f 0.88 (solvent B).

Methanolysis followed by hydrolysis. The above permethylate (250 mg) was refluxed with dry MeOH–IN HCl (50 ml) for 4 hr on a steam bath, MeOH was evaporated, H_2O (25 ml) added and hydrolysed. After the usual work up the aq. neutralized hydrolysate on PC (Solvent-E) showed the presence of 2,3,6 tri-*O*-methyl-D-glucose (R_G 0.83), 2,3 di-*O*-methyl-D-glucose (R_G 0.57), 2,3,4

tri-*O*-methyl-D-xylose (R_G 0.94) and 2,3,4,6 tetra-*O*-methyl-D-galactose (R_G 0.81).

Partial hydrolysis. Compound **1** (1 g) was refluxed on a steam bath with 5% aq HCl–MeOH (50 ml, 1:1, 45 min), neutralized (Ag_2CO_3) and filtered. The filtrate was dried under vac. and chromatographed to obtain an aglycone sarsasapogenin (Co-TLC, mp, mmp) and four prosaponins PS_1 to PS_4. Each prosaponin was acid hydrolysed and the usual work up showed only one aglycone sarsasapogenin. The aq. neutralized hydrolysates on PC (solvent D) for sugars showed D-glucose (R_f 0.18) in PS_1 and PS_2, D-glucose (R_f 0.18) and D-xylose (R_f 0.28) in PS_3, and D-glucose (R_f 0.18), and D-galactose (R_f 0.16) in PS_4.

Each prosaponin on permethylation, methanolysis followed by hydrolysis produced the following methylated sugars on PC (solvent E): PS_1, 2,3,4,6 tetra-*O*-methyl-D-glucose (R_G 1.00); PS_2, 2,3,6 tri-*O*-methyl-D-glucose (R_G 0.83); 2,3,4,6 tetra-*O*-methyl-D-glucose (R_G 1.00); PS_3, 2,3,6-tri-*O*-methyl-D-glucose (R_G 0.83), 2,3,4-tri-*O*-methyl-D-glucose (R_G 0.85), 2,3,4 tri-*O*-methyl-D-xylose (R_G 0.94); and PS_4, 2,3,6 tri-*O*-methyl-D-glucose (2 mol, R_G 0.83); 2,3,4,6 tetra-*O*-methyl-D-galactose (R_G 0.81).

Filicinin-B (**2**). Compound **2** was crystallized from MeOH. mp 181.5°, $[\alpha]_D^{20}$ $-47.5°$ (MeOH), R_f 0.40 (solvent A, 1.6 g). It was positive to Liebermann–Burchard test and negative to Ehrlich reagent test. Its IR spectrum showed well-defined characteristic spiroketal absorption bands [$920 > 900\ cm^{-1}$, 25*S*]. FAB-MS *m/z* 1203 $[M + Li]^+$.

Enzymic hydrolysis. Compound **2** (100 mg) was subjected to enzymatic hydrolysis with β-glucosidase (10 mg) as before. After 72 hr, the PC (solvent D) of the reaction mixture showed the presence of D-glucose, whereas TLC (solvent-A) showed only one spot corresponding to **1** (R_f 0.52, solvent-A), i.e. filicinin-A.

Partial hydrolysis. Compound **2** (1 g) on usual partial hydrolysis produced five prosaponins PS_5 to PS_9, of which PS_5, PS_6, PS_7 and PS_9 were found to be identical to prosaponins PS_1, PS_2, PS_3, PS_4, obtained from filicinin-A (**1**) (Co-TLC, superimposable-IR). Prosaponin PS_8 on acidic hydrolysis yielded sarsasapogenin and D-glucose (R_f 0.18, PC, solvent-D). Permethylation and methanolysis followed by hydrolysis of PS_8 provided three methylated sugars on PC (solvent E) 2,3,6 tri-*O*-methyl-D-glucose (R_G 0.83); 3,4,6 tri-*O*-methyl-D-glucose (R_G 0.84) and 2,3,4,6 tetra-*O*-methyl-D-glucose (R_G 1.00).

Filicinosides-C (**3**) and *D* (**4**). The inseparable mixture of **3** and **4** (58 g) on CC showed no spiroketal absorption bands in IR, but was positive to the Ehrlich reagent test and showed three diagonal spots in two-dimensional TLC, indicating their furostanolic nature.

Filicinoside-C (**3**). The mixture of **3** and **4** (100 mg) was refluxed with dry MeOH (50 ml) under dry conditions for 8 hr to obtain **3**; mp 179–183°, $[\alpha]_D^{20}$ $-46°$ (MeOH), R_f 0.73 (solvent F).

Filicinoside-D (**4**). The mixture of **3** and **4** (100 mg) was refluxed with aqueous Me_2CO (50 ml, 1:4) for 8 hr to yield **4**. mp 168–172°, $[\alpha]_D^{20}$ $-48°$ (pyridine), R_f 0.52 (solvent-F).

Enzymic hydrolysis. The mixture of **3** and **4** (100 mg) was taken up in H_2O (50 ml) and β-glucosidase (Sigma, 10 mg) was added followed by the addition of toluene (3 drops) to cover the aq. layer. The reaction mixture was kept at room temp. for 72 hr. The PC (solvent D) and TLC (solvent A) showed the presence of D-glucose (R_f 0.18) and a prosaponin (positive to Ehrlich Reagent, R_f 0.52) Filicinin-A(**1**), respectively.

Partial hydrolysis. The mixture of **3** and **4** was subjected to partial hydrolysis to produce five prosaponins PS_{10} to PS_{14}. Of these prosaponins PS_{12} was positive to the Ehrlich reagent test, indicating its furostanolic nature, and was found to be identical to filicinoside-B (Co-TLC, IR), whereas other prosaponins PS_{10}, PS_{11}, PS_{13}, PS_{14} were found to be identical to PS_1, PS_2, PS_3 and PS_4, respectively.

Acknowledgements—The authors are highly thankful to the Director, CDRI, Lucknow for IR, EIMS, FAB-MS and ^{13}C-NMR spectra.

REFERENCES

1. Sharma, S. C., Sati, O. P. and Chand, R. (1982) *Phytochemistry* **21**, 1711.
2. Sharma, S. C., Chand, R. and Sati, O. P. (1982) *Phytochemistry* **21**, 2075
3. Sharma, S. C., Chand, R., Bhatti B. S. and Sati, O. P. (1982) *Planta Med.* **46**, 48.
4. Sharma, S. C., Sati, O. P. and Chand, R. (1983) *Planta Med.* **47**, 117.
5. Sharma, S. C. and Kumar, R. (1983) *IUPAC Conference.* Cologne (Germany). P-190, 324.
6. Sharma, S. C. and Sharma, H. C. (1984) *Phytochemistry* **23**, 645.
7. Sati, O. P. and Sharma, S. C. (1985) *Pharmazie* **40**, 417.
8. Sharma, S. C. and Sharma, H. C. (1993) *Phytochemistry* **33**, 683.
9. Kirtikar, K. R. and Basu, B. D. (1975) *Indian Medicinal Plants* (Period, Expt., Delhi) **IV**, 2498.
10. Sharma, S. C. and Thakur, N. K. (1994) *Phytochemistry* **36**, 469.
11. Ding, Y. and Yang, C. R. (1990) *Yaoxue Xuebao* **25**, 509.
12. Kiyosawa, S. and Hutoh, M. (1968) *Chem. Pharm. Bull.* **16**, 1162.
13. Tschesche, R., Seidel, L., Sharma, S. C. and Wulff, G. (1972) *Chemische Berichte* **105**, 3397.
14. Kawasaki, T., Komori, T., Miyahara, K., Nohara, T., Hosokawa, I., Ichiro, M. and Mihoshi, K. (1974) *Chem. Pharm. Bull.* **22**, 2164.
15. Dragalin, I. P. and Kintya, P. K. (1975) *Phytochemistry* **14**, 1817.
16. Liebermann, G. (1885) *Ber. Deut. Chem. Ges.* **18**, 1804.
17. Burchard, H. (1890) *Chem. Zentbl.* **1**, 25.
18. Konishi, T., Kiyosawa, S. and Shoji, J. (1985) *Chem. Pharm. Bull.* **33**, 591.

19. Maćek, K. (1963) *Paper Chromatography* (Hais, I. M. and Maćek, K., eds), p. 289. Academic Press, New York.
20. Gulbaran, E. (1964) *J. Am. Leather Chem. Ass.* **59**, 619.
21. Scoddast, R. W., Barrett, A. J. and Northcote, D. H. (1967) *J. Biochem.* **102**, 194.
22. Tschesche, R., Lüdke, G. and Wulff, G. (1967) *Tetrahedron Letters* 2785.
23. Kiliani, H. (1930) *Berichte* **63**, 2866.
24. Klyne, W. (1950) *Biochem. J.* **47**, 41.
25. Hakomori, S. (1964) *J. Biochem.* **55**, 205.
26. Kuhn, R. and Low, I (1963) *Liebigs Ann. Chem.* **669**, 183.
27. Tschesche, R., Inchaurrondo, F. and Wulff, G. (1964) *Liebigs Ann. Chem.* **680**, 107.
28. Hensens, O. D. and Lewis, K. G. (1965) *Tetrahedron Letters* **51**, 4639.