assay was carried out ×5. While some of the dishes were exposed to diffuse light at 25°, the rest were covered with black paper and kept in darkness at the same temp. Subsequent expts were carried out with solns of concn 1, and 0.1 ppm. Some expts were carried out with a suspension of 2.5 mg psoralen in 100 ml. To observe the effect of light, the test dishes were exposed to 323, 460 and 638 lx respectively at 25°.

Acknowledgements—Our thanks are due to Prof. S. K. Mukherjee, Director, Bose Institute and Prof. A. K. Barua, Head of the Department of Chemistry, Bose Institute for their interest in the work.

REFERENCES

1. Cole, R. S. (1970) *Biochim. Biophys. Acta* **217**, 30.
2. Pathak, M. A. and Kramer, D. M. (1969) *Biochim. Biophys. Acta* **195**, 197.
3. Musajo, L. and Rodighero, G. (1962) *Experientia* **18**, 153.
4. Cole, R. S. and Zusman, D. (1970) *Biochim Biophys Acta* **224**, 660.
5. Chakraborty, D. P., Dasgupta, A. and Bose, P. K. (1957) *Ann. Biochem. Expt. Med.* **17**, 59.
6. Chakraborty, D. P., Sen, M. and Bose, P. K. (1961) *Trans. Bose Res. Inst.* **24**, 31.
7. Chakraborty, D. P. (1966) *Ind. J. Dermatol.* **11**, 1.
8. Chakraborty, D. P. (1968) *Sci. Cult.* **34**, 33.
9. Dey, R. N. (1975) Ph.D. Thesis, Calcutta University.
10. Shunichi Fucushi (1960) *Nippon Noeikagaker Kaishi* **34**, 498; *Chem. Abstr.* (1963) **58**, 11903f.
11. Baskin, J. M., Ludlow, C. J., Harris, T. K. and Wolf, F. T. (1967) *Phytochemistry* **6**, 1209.
12. Mayer, A. M. and Evenari, M. (1952) *J. Exp. Botany* **3**, 246.
13. Berrie, A. M. M., Parken, W., Knights, B. A. and Hendrie, M. R. (1968) *Phytochemistry* **7**, 567.
14. Audus, L. J. and Quastel, J. H. (1947) *Nature* **159**, 320.

Phytochemistry, 1976, Vol. 15, pp. 2006–2008. Pergamon Press. Printed in England

STILBENE GLUCOSIDES IN THE BARK OF *PICEA SITCHENSIS*

MASAKAZU ARITOMI and DERVILLA M. X. DONNELLY
Department of Chemistry, University College, Dublin 4, Ireland

(*Received* 15 *April* 1976)

Key Word Index—*Picea sitchensis*; Pinaceae; spruce; glucosides; prenylation reactions.

Abstract—The fresh bark of *Picea sitchensis* contains astringin as the major stilbene; isorhapontin and piceid are present in minor amounts. The aglycones astringenin, isorhaponhgenin and resveratrol are absent in samples of fresh bark. Prenylation reactions on 5,3′,4′-tri-*O*-methylastringenin are described.

INTRODUCTION

The bark from *Picea sitchensis*, a waste product of the pulp and paper industry, was examined for a quantitative assessment of the compounds of economic importance. Our initial interest was in the occurrence of stilbenes, a group of phenolic compounds considered [1] as the possible inhibitors of some forest pathogens (e.g. *Fomes annosus*), the control of which plays an important role in the economic cultivation of spruce forests.

RESULTS AND DISCUSSION

The dried fresh spruce bark yielded 6% astringin; other than UV spectra [2, 3] and GLC behaviour [4] no physical data for the unstable and easily oxidized 3,5,3′,4′-tetrahydroxystilbene 3-β-D-glucopyranoside is recorded. The structure of the isolate from *P. sitchensis* was confirmed by acidic hydrolysis of the methylated derivative *trans*-5,3′,4′-tri-*O*-methylastringin. The aglycone from the hydrolysate was ethylated and subsequently oxidized to give an equimolar mixture of 5-ethoxy-3-methoxybenzoic and 3,4-dimethoxybenzoic acids. The name astringin was originally proposed by Hillis to describe an amorphous mixture (mp 75–125°) isolated from *Eucalyptus sideroxylon* [5].

The minor phenolic components of the bark were isolated using $Ph(OAc)_2$ treatment. Isorhapontin was found to be present in 0.4% yield and was characterized as its hexaacetate [6]. Also obtained was the stilbene glucoside, piceid (0.08% yield) which had not previously been found in *P. sitchensis*. The structure of piceid was confirmed by isolation of resveratrol-3,4′-dimethyl ether, on hydrolysis of the methylated glucoside.

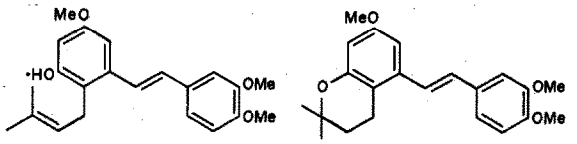
(+)-Catechin (0.08%) and sitosterol (0.01%) were also identified in the bark extract.

Chromatograms of Sitka spruce bark extracts were reported [2, 7] as showing 15 fluorescent spots, which, though the compounds were not isolated, were inferred from R_f values to be *trans*- and *cis*-stilbene glucosides and their corresponding aglycones. In the fresh bark extracts of *P. sitchensis* analysed by us, the aglycones were absent.

The presence of stilbenes and their glucosides has also been observed in the barks of *P. abies* L (Karst) [8–10]; *P. glauca* (Moench) Voss [2, 10]; *P. marianna* (Mill) B.S.P., *P. rubens*, *P. engelmannii* (Parry) [2, 11]; *P. obovata* (Ledebour) [12].

The instability of astringin in air prohibited its use in the standard antifungal and antibacterial tests, and the preparation of stable derivatives of the glucoside and its aglycone was undertaken.

Dihydroastringin was prepared by direct hydrogenation of astringin and was characterized as its heptaacetate. A series of prenylated astringenin OMe derivatives resulted from the reaction of 3-hydroxy-5,3′,4′-trimethoxystilbene with 2-methyl-3-buten-2-ol in BF_3–Et_2O. 3-Hydroxy-5,3′,4′-trimethoxy-2-prenylstilbene (**1**) and 3-hydroxy-5,3′,4′-trimethoxy-6-prenylstilbene were separated and characterized by methylation when they gave identical products. Oxidative cyclization of compound (**1**) with DDQ afforded the chromene (**2**). The third isomer from the prenylation reaction of an impure sample of 3-hydroxy-5,3′,4′-trimethoxy-4-prenylstilbene was contaminated with the 2-prenyl isomer. The first natural prenylated stilbene was reported recently [13] as occurring in *Derris rariflora*.



1 2

EXPERIMENTAL

Mp's were determined using a Kofler hot stage apparatus. IR spectra were recorded from KBr discs, UV spectra were determined in MeOH and 60 MHz PMR spectra from solns in $CDCl_3$ (TMS as internal reference). Only significant bands in IR and NMR spectra are quoted. During isolation processes, the appropriate combination of fractions were determined from TLC. Solvent systems for development were: (A) $CHCl_3$–MeOH–H_2O, (7:3:1) (lower layer); (B) $CHCl_3$–MeOH, (i) (9:1), (ii) (4:1); (C) C_6H_6–Me_2CO (9:1); (D) *n*-hexane–Me_2CO (4:1); (E) petrol (bp 40–60°)–Me_2CO (9:1).

Extraction of bark. Milled fresh bark of *P. sitchensis* (Bong.) Carr (1.5 kg) was extracted successively with *n*-hexane (48 hr), C_6H_6 (48 hr), Et_2O (72 hr) and EtOAc (48 hr). Evaporation of solvents afforded the extracts *n*-hexane (58 g, residue prior to evap. 1.2 g), C_6H_6 (6.86 g) Et_2O (10.7 g), residue prior to evaporation (11.5 g) and EtOAc (186 g). An aliquot (1 g) of the EtOAc fraction in Me_2CO was subjected to column chromatography on Sephadex LH-20 (100 g) with the eluants Me_2CO (200 ml) and Me_2CO–MeOH (4:1). The fractions showing a single spot (R_f 0.12 solvent A) were collected and crystallized from H_2O when astringin separated as needles mp 231–232° (decomp.); $[\alpha]_D^{24}$ − 65.23° (*c*, 0.21 MeOH). (Found: C, 59.16; H, 5.47. $C_{20}H_{22}O_9$ requires: C, 59.11; H, 5.46.) λ_{max}(nm) (log ε) 327 (4.43) 221 (4.37) 305 (4.33) infl. Astringin heptaacetate mp 152–153° (needles from MeOH) (lit. [13] mp 100–115°; lit. [9] mp 148–149°); $[\alpha]_D^{23}$ − 15.56° (*c* 2, $CHCl_3$) λ_{max}(nm) (log ε) 310 (4.5) 299 (4.5) 230 (4,26) 208 (4.32); PMR: δ 2.08 (12 H, *s*, OAc of sugar), 2.33 (9 H, *s*, OAc).

5,3′,4′-Tri-O-methylastringin. Astringin (500 mg) was methylated (CH_2N_2). The tri-*O*-methyl ether was purified by TLC (solvent B) and afforded needles (330 mg) (from MeOH) mp 161–162°; $[\alpha]_D^{24°}$ − 55° (*c* 0.2, MeOH). (Found: C, 59.6; H, 6.29. $C_{23}H_{28}O_9 \cdot H_2O$ requires: C, 59.22, H, 6.48%.) The tetraacetate of the tri-*O*-methyl ether was an oil. PMR: δ 2.08, 2.10 (12 H, *s*, OAc of sugar) 3.87, 3.93, 3.97 (9 H, *s*, OMe) ≈4.3, 5.0 (*m*, sugar protons), 6.56 (1 H, *t*, *J* 3 Hz, 4-H) 6.84 (2 H, *d*, *J* 3 Hz 2, 6-H) 7.0–7.8 (*m*, 2′, 5′, 6′, α and β-H).

Hydrolysis of tri-O-methylastringin. The tri-*O*-methyl ether (218 mg) in MeOH (10 ml) containing 1% HCl was refluxed for 5 hr. Hydrolysate was diluted with H_2O and extracted with Et_2O. Et_2O extractive (128 mg) was chromatographed on TLC (solvent C). Recrystallization of the major band from C_6H_6–hexane (1:1) gave 5,3′,4′-trimethoxy-3-hydroxystilbene as needles (84%), mp 124°. (Found: C, 71.59; H, 6.24. $C_{17}H_{18}O_4$ requires: C, 71.31, H, 6.34%.) λ_{max}(nm) (log ε) 323 (4.51) 305 (4.41) *sh*. PMR: δ 3.84, 3.92, 3.97 (9 H, 3 × *s*, OMe), 5.91 (1 H, *br s*, OH exchanges D_2O), 6.45 (1 H, *t*, *J* 3 Hz 4-H) 6.71 (2 H, *d*, *J* 3 Hz, 2, 6-H) 7.0–7.2 (5 H, *m*, 2′, 5′, 6′-, α- and β-H). The *monoacetate* was an oil. PMR: δ 2.33 (3 H, *s*, OAc). The aq. layer from Et_2O extraction was neutralized on amberlite IR 45. The residue, after evaporation of solvent was chromatographed on cellulose and developed 2× with *n*-BuOH–C_5H_5N–H_2O (3:2:1). The R_f of the single spot corresponded to D-glucose. 5,3′,4′-trimethoxy-3-hydroxystilbene (27 mg) was methylated (CH_2N_2–Et_2O). The residue, on evaporation, was purified by TLC (solvent C). Crystallization from MeOH gave needles of 3,5,3′,4′-tetramethoxystilbene mp 66–67° (lit. [5] 64–66°), λ_{max}nm (log ε) 323 (4.37) 302 infl. (4.31). PMR: δ 3.88 (6 H, *s*, OMe) 3.96, 4.00 (6 H, 2 × *s*, OMe) 6.6 (1 H, *t*, *J* 3 Hz 4-H) 6.78 (2 H, *d*, 3 Hz 2, 6-H).

5-Ethoxy-3,3′,4′-trimethoxystilbene. Ethylation of the tri-*O*-methylaglycone (27 mg), (C_2H_5I (0.5 g)–K_2CO_3 (1 g)–Me_2CO (20 ml)) and subsequent purification by TLC (solvent C), gave a fraction which crystallized from aq. MeOH as needles mp 58–59°. (Found: C, 72.52; H, 7.14. $C_{19}H_{22}O_4$ requires: C, 72.59; H, 7.05%.) PMR: δ 1.45 (3 H, *t*, *J* 8 Hz $OCH_2\underline{CH_3}$) 3.79, 3.95, 4.0 (9 H, 3 × *s*, OMe) 4.13 (2 H, *q*, *J* 8 Hz, $O\underline{CH_2}Me$) 6.49 (1 H, *t*, *J* 3 Hz, 4-H) 6.75 (2 H, *d*, *J* 3 Hz, 2, 6-H).

Oxidation of 5-ethoxy-3,3′,4′-trimethoxystilbene. Stilbene (653 mg) in HOAc (30 ml) was reacted with CrO_3 (0.9 g) in HOAc (50%) at 50°. The reaction mixture was stirred for 2 hr at 50–60°. Et_2O extract of the diluted reaction mixture was evaporated and residue methylated (CH_2N_2). Separation (solvent D) gave 2 fractions. Fraction (R_f 0.38) (60 mg) was hydrolyzed (MeOH–KOH, 1%) to yield 3,4-dimethoxybenzoic acid as needles (from C_6H_6 *n*-hexane) mp and mmp 179–180°. Fraction (R_f 0.63) (10 mg) was identical with an authentic sample of methyl 5-ethoxy-3-methoxybenzoate.

3-Ethoxy-5-methoxy benzoic acid. Me 3-hydroxy-5-methoxybenzoate was ethylated (EtI–K_2CO_3–Me_2CO). The reaction mixture was refluxed for 5 hr, cooled, filtered and evaporated. Residue was refluxed with MeOH–KOH for 1 hr and the acid crystallized (dil MeOH) as needles mp 142°. (Found: C, 61.5; H, 5.87. $C_{10}H_{12}O_4$ requires: C, 61.2; H, 6.1%.)

Isolation of minor constituents from the EtOAc extract. An aliquot of the EtOAc extract was treated with $Pb(OAc)_2$ in MeOH. The ppt. was collected, suspended in MeOH through which H_2S was bubbled, and the PbS discarded. Residue from the filtrate on crystillization from H_2O yielded astringin (3.2 g). The aq. mother liquor was extracted with *n*-BuOH. Residue (2.14 g) from evaporation of this extract, was chromatographed on Sephadex LH-20 and eluted with Me_2CO–MeOH (4:1). The fractions were analysed by TLC (Solvent A). Fraction 1 (0.4 g) was subjected to TLC (Solvent B (ii)) and afforded (+)-catechin (62 mg; 0.08% dry bark basis) crystallized from H_2O as needles mp 172–173°. $[\alpha]_D^{22}$ + 3.0° (*c*, 0.2 MeOH) (identical mp, NMR, UV (+)-catechin). Filtrates from $Pb(OAc)_2$ treatments were subjected to H_2S gas, the PbS discarded and the solvents evaporated. An aliquot (1 g) of the residue (1.63 g) was chromatographed on Sephadex LH-20 (100 g) and eluted with Me_2CO and Me_2CO–MeOH (4:1). Seven fractions (i–vii) were collected, of which fractions (ii–iv) yielded intractable oils. Fraction (i) (20 mg) plates from MeOH mp 136–137° (identical mp TLC, GLC β-sitosterol). Fraction (v) (1.03 g) was again treated with $Pb(OAc)_2$. Residue from the aq. filtrate was subjected to TLC (Solvent B (ii)). The band (R_f 0.3) was eluted with Me_2CO and evaporation of solvent gave a pale yellow powder (0.35 g). Acetylation (C_5H_5N–Ac_2O) afforded isorhapontin hexaacetate which was crystallized (MeOH) as needles (0.41 g) mp and mmp 160–161° (lit. [7] mp 162°). Methylation (CH_2N_2–Et_2O) of an aliquot of the yellow powder and purification by TLC (Solvent B (i)) afforded isorhapontin Me ether mp 150°, identical with 5,3′,4′-tri-*O*-methylastringin. Fraction (vi) (1.13 g) was chromatographed on Sephadex LH 20 (100 g) and eluted with Me_2CO–MeOH (4:1). One fraction R_f 0.25 (Solvent B (ii)) afforded a solid (0.13 g). A similar solid was separated from fraction (vii). These combined solids crystallized from H_2O

as pale yellow needles (0.14 g), mp 130–140°; resolidified at 170° and remelted at 228–230°. $[\alpha]_D^{19}$ − 65.26° (*c*, 0.19 MeOH) λ_{max}(nm) (log ε) 320 (4.44) 306 (4.45). Acetylation (C_5H_5N–Ac_2O) afforded piceid hexaacetate, mp 157–158° (needles from MeOH) (lit. [14] mp 157°). PMR: δ 2.08, 2.09, 2.11 (12 H, *s*, OAc on sugar moiety), 2.34 (6 H, *s*, OAc) 6.75 (1 H, *t*, *J* 2 Hz 4-H) 7.04, 7.09 (4 H, 2-, 6- α- and β-H) afforded resveratrol as needles from MeOH–H_2O mp 251–252° decomp. (lit. [14] 263°); triacetate mp 112° (lit. [14] mp 112°).

Dihydroastringin. Hydrogenation of astringin (500 mg) with Pd–C catalyst (500 mg; 5%) in MeOH (30 ml) afforded an oil which was chromatographed (Si gel; 50 g) using eluent (A). The fraction (R_f 0.29) was collected and evaporated to afford a syrup. PMR: δ (TFA.) 2.96 (4 H, *s*, $-CH_2-CH_2-$) 3.79 (1 H, *s*, OH). Acetylation (C_5H_5N–Ac_2O) gave a heptaacetate which crystallized from MeOH as needles mp 123–124°; $[\alpha]_D^{22}$ − 3.7° (*c*, 0.19, $CHCl_3$). (Found: C, 57.9; H, 5.49. $C_{34}H_{38}O_{16}$ requires: C, 58.11; H, 5.45%.) PMR: δ 2.07 (12 H, *s*, Ac sugar moiety) 2.30 (9 H, *s*, OAc) 2.93 (4 H, *s*, CH_2-CH_2); λ_{max} (log ε) 267 (3.18) 271 (3.16) *sh*.

Prenylation of 3-hydroxy-5,3′,4′-trimethoxystilbene. To a mixture of 3-hydroxy-5,3′,4′-trimethoxystilbene (1.43 g; 5×10^{-3} mol) in dry dioxane (25 ml) and BF_3–Et_2O (1.07 g; 7.5×10^{-3} mol) was added with stirring, a soln of 2-methyl-3-buten-3-ol (0.65 g; 7.5×10^{-3} mol) in dry dioxane (5 ml). The reaction mixture was allowed to stand for 12 hr, diluted and extracted with Et_2O. The residue, on evaporation of the Et_2O, showed on TLC 6 components (R_f 0.82, 0.79, 0.66, 0.58, 0.51 and 0.47; Solvent C; R_f starting material 0.47). Column chromatography of the residue (eluent C_6H_6–Me_2CO (49:1) yielded 4 fractions. Fraction (i) (946 mg) was a complex mixture of 4 components. Fraction (ii) (165 mg) crystallized from C_6H_6–petrol (60–80°) to afford 3-hydroxy-5,3′,4′, trimethoxy-2-prenylstilbene as needles (76 mg) mp 142–144°, (Found: C, 74.59; H, 7.23. $C_{22}H_{26}O_4$ requires: C, 74.55; H, 7.39%.) λ_{max} 3.21 nm (log 4.43). PMR: δ 1.77, 1.87 (6 H, *br s*, *gem* Me) 3.51 (2 H, *br d*, *J* 8 Hz, Ar–C<u>H</u>$_2$), 3.85, 3.96, 3.99 (9 H, *s*, OMe) 5.36 (1 H, *m*, C<u>H</u>=C(Me)$_2$) 6.46, 6.84 (2 H, *q*, *J* 3 Hz, 4-H, 6-H) 7.0–7.5 (5 H, *m*, 2′, 5′, 6′-H, α- and β-H). Fraction (iii) (184 mg) crystallized from $CHCl_3$– petrol (60–80°) to give 3-hydroxy-5,3′,4′-trimethoxy-6-prenylstilbene as needles (90 mg) mp 170–171°. (Found: C, 74.34; H, 7.28. $C_{22}H_{24}O_4$ requires: C, 74.55; H, 7.39%.) λ_{max} 320 nm log ε 4.38. PMR: δ 1.72, 1.85 (6 H, *s*, *gem* Me) 3.49 (2 H, *br d J* Hz Ar C<u>H</u>$_2$–) 3.84, 3.96, 3.98 (9 H, *s*, OMe), 5.36 (1 H, *m*, –C<u>H</u>=C(Me)$_2$) 6.47, 6.78 (2 H, *q J* 2 Hz 2- and 4-H) 7.0 ~ 7.4 (5 H, *m*, 2′, 5′, 6′-H and α-, β-H). Fraction (iv) (250 mg) was crystallized from $CHCl_3$–petrol (60–80°) and afforded needles of 3-hydroxy-5,3′,4′-trimethoxystilbene (79 mg) mp 122–123°.

Methylation of fractions (ii) (50 mg) *and* (iii) (50 mg). The product from the individual methylations (MeI (500 mg)–K_2CO_3 (500 mg)–Me_2CO (10 ml) afforded 3,5,3′,4′-tetramethoxy-2-prenylstilbene as an oil (55 mg)). PMR: δ 3.87, 3.89, 3.95, 3.99 (12 H, *s*, OMe).

5,3′,4′-Trimethoxy-2″,2″-dimethylpyrano (5″, 6″; 2, 3) *stilbene* (**2**). To 3-hydroxy-5,3′,4′-trimethoxy-2-prenylstilbene (124 mg) in dry C_6H_6 (12 ml) was added a soln of DDQ (80 mg) in C_6H_6 (8 ml) and the mixture was refluxed for 2 hr. Filtration and evaporation of the filtrate gave a residue which distilled (bp 198–200°/0.5 mm Hg) as a pale yellow oil. Purification by TLC (Solvent E) afforded two spots on TLC (R_f 0.28 and 0.44). Crystallization from petrol (60–80°) of the residue from band R_f 0.28 gave 5,3′,4′-trimethoxy-2″,2″-dimethylpyrano (5″, 6″; 2, 3) stilbene as needles (61% yield) mp 128–129°. (Found: C, 75.33; H, 6.84. $C_{22}H_{24}O_4$ requires: C, 74.97; H, 6.86%.) λ_{max} ($CHCl_3$) nm (log ε) 330 (4.39) 293 (4.32) PMR: δ 1.48 (6 H, *s*, Me) 3.87, 3.95, 3.99 (9 H, *s*, OMe) 5.65 (1 H, *d*, *J* 10 Hz, 3″-H) 6.45, 6.77 (2 H, *q*, *J* 2.5 Hz 2-, 4-H) 6.78 (1 H, *d*, *J* 10 Hz 4″-H) 7 ~ 7.5 (5 H, *m*, 2′, 5′, 6′- and α-, β-H).

Acknowledgements—We thank the National Science Council of Ireland for the award of a Fellowship (to M.A.); to Dr. J. Dunleavy, Forest Products Department, IIRS Dublin for the collection and preparation of bark samples; to Dr. E. P. Swan, Canadian Forestry Service, Vancouver and Dr. A. Yagi, Kyushu University for samples of stilbenes.

REFERENCES

1. Alcubilla, Von M., Diaz-Palacio, M. P., Kreutzer, K., Laatsch, W., Rehfuess, K. E. and Denzel, G. (1971) *European J. For. Path.* **1**, 100.
2. Manners, G. D. and Swan, E. P. (1971) *Phytochemistry* **10**, 607.
3. Hillis, W. E. and Ishikura, N. (1968) *J. Chromat.* **32**, 323.
4. Hemingway, R. W., Hillis, W. E. and Bruerton, K. (1970) *J. Chromat.* **50**, 391.
5. Hillis, W. E. and Hasegawa, Masao (1962) *Biochem. J.* **83**, 503.
6. Andrews, D. H., Hoffman, F. C., Purves, C. B., Quon, H. H. and Swan, E. P. (1968) *Can. J. Chem.* **46**, 2525.
7. Hergert, H. L. (1960) *Forest Prod. J.* **10**, 610.
8. (a) Grassmann, W., Deffner, G., Schuster, E. and Pauckner, W. (1956) *Chem. Ber.* **89**, 2523; (b) Grassmann, W., Endres, H. and Pauckner, W. (1958) *Chem. Ber.* **91**, 134; (c) Grassmann, W. and Endres, H. (1965) *J. Chem. Soc.* 4579.
9. Cunningham, J., Haslam, E. and Haworth, R. D. (1963) *J. Chem. Soc.* 2875.
10. Alcubilla-Martin, M. (1970) *Landwirt. Forsh. Sonderh.* **25**, 96.
11. Manson, D. W. (1960) *Tappi* **43**, 59.
12. Shibanova, G. I. and Pentagava, V. A. (1970) *Isv. Vyssh. Uch. Zav. Tech. Li. Prom.* 58.
13. Braz Fo, R., Gottlieb, O. R. and Mourao, A. P. (1975) *Phytochemistry* **14**, 261.
14. Hillis, W. E. and Isai, K. (1965) *Phytochemistry* **4**, 541.
15. Kariyone, T., Takahashi, M., Ito, T. and Masutani, K. (1958) *Yakugaku Zasshi* **78**, 935.