Acknowledgements—We thank Dr. Scott A. Mori and Dr. P. Alvim, Herbario Centro de Pesquisas do Cacau at Itabuna, Bahia, Brazil, for their help during plant collection, and the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

1. Bohlmann, F., Burkhardt, T. and Zdero, C. (1973) *Naturally Occurring Acetylenes*. Academic Press, London.
2. Bohlmann, F. and Grenz, M. (1970) *Chem. Ber.* **103**, 90.
3. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1980) *Phytochemistry* **19**, 1141.
4. Gorinsky, C., Templeton, W. and Zaidi, S. A. H. (1973) *Lloydia* **36**, 352.
5. Czerson, H., Bohlmann, F., Stuessy, T. F. and Fischer, N. H. (1979) *Phytochemistry* **18**, 257.
6. Stuessy, T. F. (1977) *The Biology and Chemistry of the Compositae* (Heywood, V. H., Harborne, J. B. and Turner, B. L., eds.). Academic Press, London.

Phytochemistry, 1980, Vol. 19, pp. 2501–2503. © Pergamon Press Ltd. Printed in England. 0031-9422/80/1101-2501 $02.00/0

A CHALCONE GLYCOSIDE FROM *ABIES PINDROW*

K. P. TIWARI and P. K. MINOCHA*

Department of Community Health and Environmental Medicine, Odense University, Odense, Denmark: * Department of Chemistry, University of Allahabad, Allahabad-211002, India

(*Revised received* 29 *February* 1980)

Key Word Index—*Abies pindrow*: Coniferae; 2′,3′,4′:3,4-pentahydroxy chalcone 4′-(L-arabinofuranosyl-α-1→4-D-glucopyranoside-β); permethylation; structural determination.

Abstract The EtOH extract of air-dried stems of *Abies pindrow* yielded okanin, okanin 4′-*O*-β-D-glucopyranoside, butein 4′-*O*-β-D-glucopyranoside, 8,3′4′-trihydroxyflavanone-7-*O*-β-D-glucopyranoside and a new chalcone glycoside, 2′,3′,4′:3,4-pentahydroxy-chalcone 4′-(L-arabinofuranosyl-α-1→4-D-glucopyranoside-β).

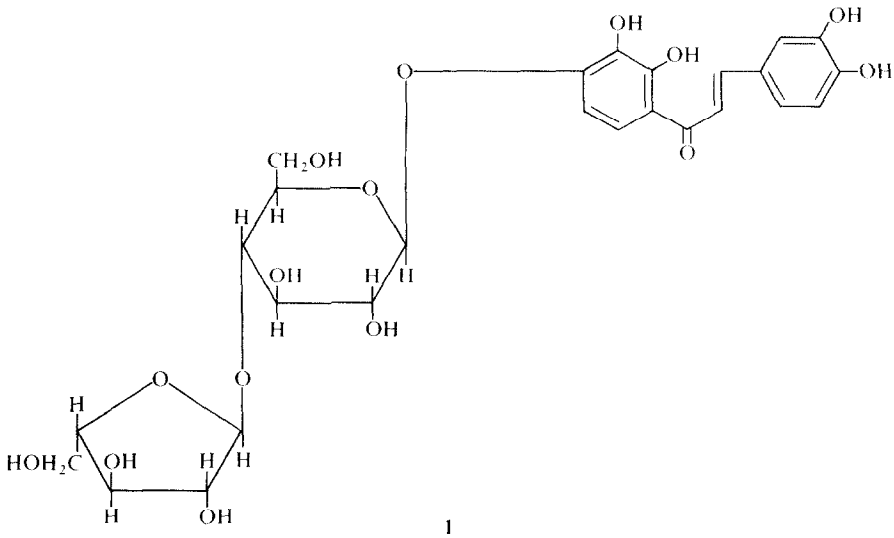
INTRODUCTION

Abies pindrow is a tall evergreen tree with a dense conical crown of dark green foliage. Several species of *Abies* are regarded as carminative, expectorant, stomachic, toxic and astringent [1]. Since no work seems to have been previously done on the chemical constituents of *A. pindrow*, we have, therefore, examined the stem of this plant and its constituents are reported in the present paper.

RESULTS AND DISCUSSION

From the EtOH extract of the stem six compounds A to F were isolated. Compound A, a chalcone, has been characterized as okanin by spectral studies, derivative formation, mmp and co-PPC with an authentic sample [2, 3]. Chalcone B could not be studied due to lack of material. Compounds C, D and E were characterized as okanin-4′-*O*-β-D-glucopyranoside, butein-4′-*O*-β-D-glucopyranoside and 8,3′,4′-trihydroxy-flavanone-7-*O*-β-D-glucopyranoside, respectively, by comparison of spectral data, mmp and co-PPC with authentic samples [4–7].

Compound F, $C_{26}H_{30}O_{15}$, gave characteristic tests for a chalcone-glycoside [8, 9]. On acid hydrolysis it yielded an aglycone, which was identical to compound A in all respects. The sugar portion on co-chromatographic examination revealed the presence of D-glucose and L-arabinose (BuOH–HOAc–H_2O, 4:1:5, R_f 0.18 and 0.21 respectively). Quantitative hydrolysis of the glycoside indicated the aglycone content was *ca* 49%. Quantitative estimation [10] of the sugars present in the hydrolysate revealed that both sugars were present in equimolecular proportions. On partial hydrolysis [11] with 0.02N H_2SO_4 for 7 days at room temperature, it yielded a glycosidic compound which was purified by column chromatography over Si gel using EtOAc as eluant. It was identical with compound C in all respects. The hydrolysate revealed the presence of L-arabinose only, indicating that it was the terminal sugar of compound F. Permethylation [12] of F, followed by hydrolysis showed the presence of 2:3:6-tri-*O*-methyl-D-glucose and 2:3:5-tri-*O*-methyl-L-arabinose (BuOH–HOAc–H_2O, 4:1:5, R_G 0.83 and 0.95, respectively) [13]. This observation suggested that arabinose was present as the furanoside and that it was attached by C-1 to C-4 of the glucose moiety. The above fact was also confirmed by periodate oxidation [14]. F on



1

enzymatic hydrolysis with diastase yielded L-arabinose only, indicating that L-arabinose is joined to glucose by an α-linkage; this was further supported by the fact that L-arabinose could not be detected by PPC after hydrolysing F with emulsin [15,16]. As glucose is attached through a β-linkage in C, which is also the partially hydrolysed product of F, the exact configuration of the sugar linkages in F was D-glucose-β and L-arabinose-α. Hence compound F is 2′,3′,4′: 3,4-pentahydroxy-chalcone-4′-(L-arabino-furanosyl-α-1→4-D-glucopyranoside-β) and may be represented by structure **1**.

EXPERIMENTAL

The plant was identified by the Botanical Survey of India, Allahabad.

Extraction and isolation. The air-dried, defatted, powdered stem of *A. pindrow* (Royal) Spach (4 kg) was exhaustively extracted with EtOH. The EtOH extract (8 l.) was concd *in vacuo* to a thick viscous mass and poured into an excess of H_2O. The coloured ppt. obtained was successively washed with petrol, C_6H_6 and $CHCl_3$ and finally with EtOAc. The EtOAc extract was concd and chromatographed over a column of Si gel. Eluates using EtOAc satd with H_2O yielded two chalcones, A and B. A (750 mg), mp 231–233°, R_f 0.56 (PC, BuOH–HOAc–H_2O, 4:1:5). (Found: C, 62.8; H, 4.19. M^+ 288 (MS). $C_{15}H_{12}O_6$ requires: C, 62.50; H, 4.16%). UV λ_{max}^{EtOH} nm: 260, 330, 382; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 271, 420; $\lambda_{max}^{EtOH+NaOEt}$ nm: 447 $\lambda_{max}^{EtOH+NaOAc+H_3BO_3}$ nm: 411. Penta Me derivative, $C_{20}H_{22}O_6$, mp 94°, B (12 mg), mp 202° could not be studied. The H_2O sol. fraction (after separating the ppt.) was concd and chromatographed over a column of magnesol. Elution with EtOAc satd with H_2O yielded 4 fractions. All four on further column chromatography over magnesol, using EtOAc as eluant, yielded compounds C to F. C (250 mg), mp 130–132°. (Found: C, 55.82; H, 4.81. $C_{21}H_{22}O_{11}$ requires: C, 56.0; H, 4.88%). UV λ_{max}^{EtOH} nm: 259, 326, 385; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 276, 329, 428; $\lambda_{max}^{EtOH+NaOEt}$ nm: 465; $\lambda_{max}^{EtOH+H_3BO_3+NaOAc}$ nm: 415. D (360 mg), mp 190–192°. (Found: C, 57.84; H, 5.12. $C_{21}H_{22}O_{10}$ requires: C, 58.06; H, 5.06%). UV λ_{max}^{EtOH} nm: 265, 305, 385; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 275, 317, 430; $\lambda_{max}^{EtOH+NaOEt}$ nm: 450; $\lambda_{max}^{EtOH+NaOAc+H_3BO_3}$ nm: 415. E (420 mg), mp 238–240°. (Found: C, 55.78; H, 4.82. $C_{21}H_{22}O_{11}$ requires: C, 56.0; H, 4.88%). UV λ_{max}^{EtOH} nm: 280, 320. Aglycone of E, $C_{15}H_{12}O_6$, mp 126–127°. tetraMe derivative, $C_{19}H_{20}O_6$, mp 143–144°. F (380 mg), mp 186–188°. (Found: C, 53.66; H, 5.12. $C_{26}H_{30}O_{15}$ requires: C, 53.60; H, 5.15%). UV λ_{max}^{EtOH} nm: 264, 324, 388. Aglycone $C_{15}H_{12}O_6$, mp 232–233°; UV λ_{max}^{EtOH} nm: 260, 330, 382.

Quantitative estimation of sugars in hydrolysate of F. The ratio of sugars was determined colorimetrically [10] in a Klett–Summerson photo-electric colorimeter using a blue filter (420 nm) with the help of standard curves of authentic sugars. Ten solns (5, 10, 15.....50 μg in 0.03 ml H_2O) of both sugars, D-glucose and L-arabinose were applied on Whatman No. 1 filter paper (50 × 55 cm, spot distance 5 cm). The chromatograms were developed descending with BuOH–HOAc–H_2O (4:1:5) for 24 hr, dried in air, sprayed with aniline hydrogen phthalate and dried at 110° for 15 min. The coloured spots were cut out in equal rectangles eluted by immersion in 50% HOAc (10 ml each) and the colour intensity of each eluate measured. The hydrolysate of F was neutralized with $BaCO_3$, filtered and concd to a syrup (1 ml). 0.2 ml of this syrup was dissolved in 2 ml of H_2O and aliquots applied to Whatman No. 1 filter paper. The chromatograms were developed, sprayed, dried and the coloured spots were cut out in equal rectangles, eluted separately and assayed as described above.

Partial hydrolysis of F. F (120 mg) was treated with 0.02 N H_2SO_4 and the reaction mixture kept at room temp. for 7 days. It was then extracted with EtOAc. The EtOAc extract after concn was chromatographed over a column of Si gel. Elution with EtOAc yielded a compound (75 mg), mp 131–132°. (Found: C, 55.92; H, 4.84. $C_{21}H_{22}O_{11}$ requires: C, 56.0; H, 4.88%). It was identical to compound C.

Permethylation of F and hydrolysis of permethylated derivative. F (50 mg) was treated with MeI (1.5 ml) and Ag_2O in DMF (3 ml) for 48 hr at room temp. The mixture was filtered and the residue washed with a little DMF. The filtrate was evapd to dryness and the residue taken up in EtOH (20 ml). The syrup obtained after removal of EtOH was hydrolysed with Kiliani's mixture (HOAc–HCl–H_2O, 7:3:10) and the product worked up in the usual way. The hydrolysate on PC examination revealed the presence of 2:3:6-tri-*O*-methyl-D-glucose and 2:3:5-tri-*O*-methyl-L-arabinose (BuOH–EtOH–H_2O, 5:1:4. R_G 0.83 and 0.95, respctively).

Periodate oxidation of F. F (40 mg) was dissolved in 15 ml EtOH and 15 ml 0.15 M Na metaperiodate soln was added. The

oxidation was allowed to take place at room temp. for 60 hr. Aliquots (5 ml) were withdrawn in duplicate from the reaction mixture at different times and analysed for periodate and formic acid.

Acknowledgement—We are grateful to Dr. S. K. Srivastava, University of Saugar, Sagar, who kindly supplied authentic samples of some of the compounds reported in this paper.

REFERENCES

1. Kirtikar, K. R. and Basu, B. D. (1933) *Indian Medicinal Plant*, Vol. 3, p. 324.
2. Geissman, T. A., Harborne, J. B. and Seikel, M. K. (1956) *J. Am. Chem. Soc.* **78**, 825.
3. Harborne, J. B. and Geissman, T. A. (1956) *J. Am. Chem. Soc.* **78**, 829.
4. Shimokoryama, M. (1957) *J. Am. Chem. Soc.* **79**, 214.
5. King, F. E. and King, T. J. (1951) *J. Chem. Soc.* 569.
6. Clark, J. W. *et al.* (1964) *Aust. J. Chem.* **17**, 1164.
7. Shimokoryama, M. and Geissman, T. A. (1960) *J. Org. Chem.* **25**, 1956.
8. Geissman, T. A. (1955) in *Modern Methods of Plant Analysis* (Peach, K. and Tracey, M. V., eds.) Vol. 3, pp. 464 and 468. Springer, Berlin.
9. Seikel, M. K. (1962) in *The Chemistry of Flavonoid Compounds* (by Geissman, T. A., ed.) pp. 51, 303. Pergamon Press, Oxford.
10. Misra, S. B. and Rao, V. K. M. (1960) *J. Sci. Ind. Res.* **19C**, 173.
11. Dutta, T. and Basu, U. P. (1968) *Indian J. Chem.* **6**, 471.
12. Kuhn, R., Trischhmann, H. and Low, I. (1955) *Angew. Chem.* **67**, 32.
13. Lederer, E. and Lederer, M. (1955) in *Chromatography*, p. 163. Elsevier, Amsterdam.
14. Hirst, E. L. and Jones, J. K. N. (1949) *J. Chem. Soc.* 1659.
15. Tiwari, K. P. and Singh, R. B. (1978) *Phytochemistry* **17**, 1192.
16. Masood, M., Pandey, A. and Tiwari, K. P. (1979) *Phytochemistry* **18**, 1539.

Phytochemistry, 1980, Vol. 19, pp. 2503–2504. Pergamon Press Ltd. Printed in England.

(2*R*,3*S*,4*S*)-3,4,7,3′,4′-PENTAMETHOXY-2,3-*TRANS*-3,4-*CIS*-FLAVAN, A NOVEL FLAVAN FROM *NEORAUTANENIA AMBOENSIS*

MARIA E. OBERHOLZER, GERHARDUS J. H. RALL and DAVID G. ROUX

Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 Republic of South Africa

(*Received* 22 *February* 1980)

Key Word Index—*Neorautanenia amboensis;* Leguminosae; [2*R*,3*S*,4*S*]-leucofisetinidin; (+)-7,3′,4′-trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol; (+)-7,3′,4′-trimethoxy-2,3-*trans*-flavan-3,4-*cis*-diol; (+)-3,4,7,3′,4′-pentamethoxy-2,3-*trans*-3,4-*cis*-flavan.

Recent investigations [1–3] of the root bark of *Neorautanenia amboensis* Schinz revealed the presence of an exceptionally complex mixture of over 50 flavonoid compounds (mainly isoflavonoids), including rotenoids, pterocarpans, a pterocarpan-6a-ol, a pterocarpene, an isoflavan-4-ol, isoflavanones, isoflavones, a 3-phenylcoumarin, benzofurans and coumarins. We now report that examination of the methanol-soluble extractives resulted in the isolation and characterization of the leucofisetinidin **1**, (+)-7,3′,4′-trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol, and two new natural products*, (+)-7,3′,4′-trimethoxy-2,3-*trans*-flavan-3,4-*cis*-diol **2** and (+)-3,4,7,3′,4′-pentamethoxy-2,3-*trans*-3,4-*cis*-flavan **3**, with identical absolute configurations.

These new products represent different degrees of methylation of leucofisetinidin **1** with the unprecedented phenomenon in **3** where the equivalent of the 3,4-diol function is fully methylated. The isolation of **1**–**3** also represents the first observation of flavonoids associated with the predominant isoflavonoids in the *Neorautanenia* family; their origins being speculatively attributed to a common intermediate along the biogenetic pathway. This, together with the high degree of methylation of isoflavonoids present in the same source, probably explains the occurrence of flavans **2** and **3**.

Initial separation of components in the methanol extract was achieved through countercurrent distribution H_2O–butan-2-ol–*n*-hexane, 5:4.5:0.5 followed by chromatography on Si gel (C_6H_6–*n*-hexane–EtOAc, in 1:1:0.1). Final separations were done by preparative TLC and crystallization.

* Satisfactory analytical, spectroscopic and mass spectrometric data have been obtained.