2. Ranganathan, K. R. and Seshadri, T. R. (1974) *Indian J. Chem.* **12**, 888.
3. Ranganathan, K. R. and Seshadri, T. R. (1974) *Indian J. Chem.* **12**, 993.
4. Sharma, D. K. (1976) Ph.D. Thesis. Delhi University, Delhi, India.
5. Hansel, R., Kaloga, M. and Pelter, A. (1977) *Tetrahedron Letters* 4547.
6. Castelao, J. F., Jr., Gottlieb, O. R., De Lima, R. A., Mesquita, A. A. L., Gottlieb, H. E. and Wenkert, E. (1977) *Phytochemistry* **16**, 735.
7. Ternai, B. and Markham, K. R. (1976) *Tetrahedron* **32**, 565; Markham, K. R. and Ternai, B. (1976) *Tetrahedron* **32**, 2607; Wagner, H., Chari, V. M. and Sonhenbichler, J. (1976) *Tetrahedron Letters* 1799.
8. The alternate formulation with interchange of substituents in the dioxane ring is also acceptable but (1) is preferred here in view of the structure of silymarin, see Hansel, R., Schulz, J. and Pelter, A. (1975) *Chem. Ber.* **108**, 1482.
9. Wenkert, E. and Gottlieb, H. E. (1977) *Phytochemistry* **16**, 1811.

Phytochemistry, 1979, Vol 18, pp. 508–510. © Pergamon Press Ltd. Printed in England 0031-9422/79/0301-0508 $02.00/0

FLAVONOIDS OF *ABIES AMABILIS* NEEDLES

WILLIAM H. PARKER*, JACK MAZE† and DAVID G. MCLACHLAN*

*Department of Biology, Lakehead University, Thunder Bay, Ontario, Canada, P7B 5E1; †Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1W5

(*Revised received* 11 *September* 1978)

Key Word Index—*Abies amabilis*; Pinaceae; flavonol glycosides; *C*-glycosylflavones; syringetin; laricytrin; dihydroquercetin.

Abstract—Seventeen flavonol glycosides were identified from needles of *Abies amabilis* and these were based on 6 aglycone types: syringetin, isorhamnetin, kaempferol, quercetin, laricytrin and myricetin. Glycosides were 3-*O*-rutinosides, 3-*O*-glucosides, 3-*O*-galactosides or 3-*O*-rhamnosides. Also identified as needle constituents were rhamnosylvitexin and dihydroquercetin.

INTRODUCTION

We have begun a series of investigations to discover patterns of evolution in North America species of *Abies* Mill (true firs). In addition to various anatomical and morphological characters of needles, twigs and cones, we are determining the needle flavonoids of all species for future use as chemotaxonomic characters. This initial study reports the leaf flavonoids of *A. amabilis* (D. Douglas) J. Forbes (Pacific silver fir) which is a common, low-evelation, coniferous species of the Cascade and Coast Mountains of Oregon, Washington and British Columbia.

Although the terpenoids from various tissues of *A. amabilis* have been well studied as a source of chemotaxonomic input [1, 2], the flavonoids of this species (and many other common conifers) have been largely neglected. Mullick [3] has reported cyanidin as a red pigment in the periderm of *A. amabilis*, and Hergert and Goldschmid [4] found the 3′-*O*-glucoside of dihydroquercetin in its wood and bark.

RESULTS AND DISCUSSION

The needles of *A. amabilis* proved to be a rich source of varied flavonoids, although these compounds were present in low concentration relative to angiosperm foliage that we have examined. Three classes of flavonoids are present: flavonol glycosides, *C*-glycosylflavones and a dihydroflavonol (see Table 1).

Table 1. R_f values for flavonoid glycosides of *Abies amabilis*

Compound	R_f (× 100) Solvent 1*	Solvent 2†
Syringetin 3-*O*-rutinoside	47	76
Kaempferol 3-*O*-rutinoside	37	42
Quercetin 3-*O*-rutinoside	39	22
Laricytrin 3-*O*-rutinoside	41	32
Isorhamnetin 3-*O*-rhamnoside	24	56
Isorhamnetin 3-*O*-galactoside	20	45
Isorhamnetin 3-*O*-glucoside	20	45
Kaempferol 3-*O*-rhamnoside	19	33
Kaempferol 3-*O*-galactoside	27	34
Kaempferol 3-*O*-glucoside	27	34
Quercetin 3-*O*-rhamnoside	17	22
Quercetin 3-*O*-galactoside	17	19
Quercetin 3-*O*-glucoside	17	19
Laricytrin 3-*O*-rhamnoside	21	32
Laricytrin 3-*O*-galactoside	21	24
Laricytrin 3-*O*-glucoside	21	24
Myricetin 3-*O*-rhamnoside	17	9
Rhamnosylvitexin	48	28
M‡	36	61
N‡	39	56
Dihydroquercetin	45	61

* Solvent 1 = H_2O–*n*BuOH–Me_2O–HOAc (16:2:2:1) on Polyamide DC 6.6.

† Solvent 2 = $CHCl_3$–*iso*PrOH–butanone–HOAc (10:3:3:4) on Polyamide DC 6.6.

‡ Partially identified as rhamnosyl-*C*-glycosyl derivatives of apigenin.

Flavonol glycosides were the most common flavonoid constituents of the needles including monosides and flavonolbiosides. (Trace amounts of flavonol aglycones were also detected on chromatograms, but these may be artifacts from air-drying of foliage in plant presses.) UV-spectral and hydrolytic procedures indicated that glycosylation occurred only at the 3-position of the flavonol nucleus. Flavonol 3-*O*-monosides include: myricetin 3-*O*-β-D-rutinosides from *Limnanthes* [5], indicates that sides and 3-*O*-galactosides of isorhamnetin, kaempferol, laricytrin (3′-*O*-methylmyricetin) and quercetin. In each case the 3-*O*-galactosides and 3-*O*-glucosides of a specific aglycone type could not be separted chromatographically, but were hydrolysed as 2-component mixtures and the sugars and aglycones identified by chromatography against standards. To the best of our knowledge, this is the first report of the naturally occurring 3-*O*-rhamnoside and 3-*O*-galactoside of laricytrin.

Hydrolysis of 4 purified flavonol 3-*O*-biosides yielded the aglycones: syringetin: kaempferol, quercetin and laricytrin, and in each case equal amounts of glucose and rhamnose. Comparison of these compounds by UV, PMR and co-chromatography with authentic flavonol 3-*O*-β-D-rutinosides from *Limnanthes* [5], indicate that the rhamnosylglycosides of *Abies* are identical.

Three additional flavonoids with chromatographic properties similar to flavonol 3-*O*-diglycosides were isolated. However, in each case mild acid hydrolysis yielded rhamnose and a UV-absorbing compound with an R_f in the aqueous solvent characteristic of a flavonoid monoside but which resisted further hydrolysis. The UV spectra of these compounds were indistinguishable from published values [6] for *C*-glycosylated derivatives of apigenin. One of the 3 compounds was isolated and crystallized in sufficient quantity for PMR of its TMSi ether and it was positively identified as rhamnosylvitexin (see PMR in Experimental). Since this compound had the lowest R_f of the 3 flavonoids in organic solvents, it is probable that one of the remaining two compounds (M and N) is rhamnosylisovitexin, although this judgement must await confirmation either by PMR or chromatography.

A further flavonoid was isolated which appeared tan on polyamide chromatograms under UV light. It resisted hydrolysis, and on the basis of its UV spectra, it was identified as dihydroquercetin. Several other flavonoid glycosides were consistently present in needle extracts, but always occurred in relatively low concentrations which prevented their identification.

The complement of needle flavonoids reported here for *A. amabilis* is similar to those reported for *Larix* spp. [7, 8] consisting of flavonol 3-*O*-rutinosides, flavonol 3-*O*-monosides, as well as vitexin derivatives. The presence of syringetin and laricytrin as common flavonol aglycone type in *Abies*, as well as *Larix*, suggests that these compounds are relatively widespread leaf constituents of the Pinaceae.

Niemann [9] has surveyed the leaves of many gymnosperms for the presence of *C*-glycosylflavones including *Abies*, *Larix*, and 6 other genera of the Pinaceae. Of these, he reported positive findings for *Larix* spp. only. Although Niemann was unable to find *C*-glycosylflavonoids in two species of *Abies*, this result is consistent with our own observations which show these compounds absent from several *Abies* spp. (Parker *et at.*, unpublished data).

Medvedeva *et al.* [10] have identified rhamnosylvitexin (which they named 'abietin') from the needles of *A. nephrolepis* and/or *A. sibirica*, an occurrence which parallels our findings for *A. amabilis*. However, in contrast to our results, Medvedeva *et al.* did not detect any glycosides based on the 'rare' flavonol aglycone types, syringetin and laricytrin. In addition, they found 7-*O*-glycosylated derivatives of the common flavonols in the Siberian firs, a glycosylation pattern which is absent from *A. amabilis*. These differences tend to substantiate taxonomic treatments [11] of *Abies* which place these species into separate sections.

EXPERIMENTAL

Foliage for chemical analysis was collected from *ca* 12 trees of *Abies ambilis* on July 18, 1975 near Terrace, British Columbia. Voucher specimens have been deposited in UBC and LKHD.

Flavonoid isolation and identification. Intact branches were first dried in field presses and later the foliage was stripped from the twigs. Air-dried needles (1.15 kg) were extracted × 3 with MeOH at 20°, the extract filtered and concd *in vacuo* at 30°. The resulting residue was triturated with Celite prior to column chromatography on Polyamide SC-6 using a gradient of 0–50% MeOH in H_2O. Column fractions were concd and further chromatographed on columns of G-25 Sephadex (20–40% Me_2CO in H_2O) or LH-20 Sephadex (30% MeOH in H_2O). The resulting column fractions were repeatedly chromatographed (TLC) on DC 6.6 Polyamide in $CHCl_3$–MeOH–butanone–H_2O (55:22:20:3), in $CHCl_3$–*iso*PrOH–butanone–HOAc (10:3:3:4), and in H_2O–*n*BuOH–Me_2CO–HOAc (16:2:1:1). Flavonoids isolated in this fashion were identified by conventional methods including UV [6], PMR TMSi ethers, acidic and enzymic hydrolyses [12, 13], and chromatographic comparisons to previously authenticated flavonoid standards [5].

PMR of rhamnosylvitexin TMSi ether. 80 MHz in $CDCl_3$ (TMS as internal reference) δ flavone protons: 7.83 (2H, *d*, $J = 9$ Hz, H-2′,6′), 6.91 (2H, *d*, $J = 9$ Hz, H-3′,5′), 6.46 (1H, *s*, H-3), 6.21 (1H, *s*, H-6); sugar protons: glucose H-1″—4.85 (1H, *d*, $J = 9.5$ Hz, glucose H-1″), 4.45 (1H, rhamnose H-1″), 0.62 (3H, rhamnose CH_3), 3.19–3.94 (10H).

Acknowledgements—This research was supported by the National Research Council of Canada Operating Grants A0159 and A3747 and a portion of a Canadian Forestry Service Subvention Fund Grant made to the School of Forestry, Lakehead University. We thank Ms. Jane Ekholm for technical assistance and Dr. Tom Griffiths for running the PMR spectra.

REFERENCES

1. Zavarin, R., Snajberk, K. and Critchfield, W. B. (1973) *Biochem. Syst.* **1**, 87.
2. von Rudloff, E. and Hunt, R. S. (1977) *Can. J. Botany* **55**, 3087.
3. Mullick, D. B. (1969) *Can. J. Botany* **47**, 1419.
4. Hergert, H. L. and Goldschmid, O. (1958) *J. Org. Chem.* **23**, 700.
5. Parker, W. H. and Bohm, B. A. (1975) *Phytochemistry* **14**, 553.
6. Marbry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Heidelberg.
7. Niemann, G. J. (1973) *Phytochemistry* **12**, 2056.
8. Tyukavkina, N. A., Medvedeva, S. A. and Ivanova, S. Z. (1974) *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk.* 111.
9. Niemann, G. J. and Miller, H. J. (1975) *Biochem. Syst. Ecol.* **2**, 169.
10. Medvedeva, S. A., Tyukavkina, N. A. and Ivanova, S. Z.

(1974) *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk.* 111.
11. Liu, T. S. (1971) *A Monograph of the Genus* Abies. Dept of Forestry, College of Agriculture, National Taiwan Univ., Taipei, Taiwan.
12. Collins, F. W. and Bohm, B. A. (1974) *Can. J. Botany* **52**, 307.
13. Wilkins, C. K. and Bohm, B. A. (1976) *Can. J. Botany* **54**, 2133.

Phytochemistry, 1979, Vol. 18, pp 510–511. Pergamon Press Printed in England.

TWO NEW QUERCETIN SULPHATES FROM LEAVES OF *FLAVERIA BIDENTIS*

JOSÉ L. CABRERA and HÉCTOR R. JULIANI

Cátedra de Farmacognosia, Dpto. de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Estafeta 32, 5000 Córdoba, Argentina

(*Revised received* 21 *September* 1978)

Key Word Index—*Flaveria bidentis* var. *angustifolia*; Compositae; quercetin 3,4′-disulphate and 3,7,4′-trisulphate.

In previous flavonoid studies of the genus *Flaveria*, three flavonol derivatives with a high degree of sulphation: isorhamnetin 3,7-disulphate; quercetin 3-acetyl-7,3′4′-trisulphate and quercetin 3,7,3′,4′-tetrasulphate were isolated [1–3]. The present work reports two new quercetin sulphate derivatives: 3,4′-disulphate (**I**) and 3,7,4′-trisulphate (**II**).

EXPERIMENTAL

Plant source. The leaves of *Flaveria bidentis* var. *angustifolia* O. Kuntze were collected in the neighbourhood of the Ciudad Universitaria (Córdoba, Rep. Argentina) during February and March, and identified by Prof. Ing. Agr. Armando T. Hunziker (Botanical Museum, National University of Córdoba).

Isolation. 350 g of leaves were dried, ground and extracted with (1) petrol, (2) CH_2Cl_2 and finally with EtOH–H_2O (1:1). The latter extract was concd and a crystalline ppt. (6 g) was obtained by the addition of EtOH. After recrystallization from H_2O, the solid was analysed electrophoretically and chromatographically on Whatman 3 MM paper using the conditions and solvent systems shown in Table 1. By this means four compounds were visualized under UV light, which were separated on a Sephadex G-10 column (23 × 2 cm, 200 mg each time) eluting with H_2O. Quercetin and isorhamnetin 3-sulphates were identified by standard procedures and co-chromatography with authentic samples.

Compound **I**. Chromatographic and electrophoretic data are given in Table 1, mp 285° (dec). Acid hydrolysis gave quercetin (mmp, co-PC and UV data) and sulphate (a white ppt. with $BaCl_2$). UV λ_{max} (nm) in EtOH–H_2O (1:1): 247 sh, 266 and 334; +NaOMe: 272, 371 with decrease in intensity; +NaOAc: 272 and 370; +$AlCl_3$ + HCl: 274,345 and 400. IR (KBr disc) ν_{max} cm^{-1}: 3350 (OH), 1640 (CO), 1250 and 1040 (SO). NMR (DMSO-d_6, 60 MHz): δ 5.9 (1H, *d*, $J_{6,8}$ = 2 Hz, C6); 6.1 (1H,*d*, $J_{8,6}$ = 2 Hz, C8); 7.05 (1H, *d*, $J_{5',6'}$ = 9 Hz, C5′) and 7.40 (2H, *br*, C2′, 6′). (Found: S, 11.27%. Cal. for $C_{15}H_8O_{13}S_2K_2$: S, 11.91%). The electrophoretic, spectral properties and dark colour (UV/+HN_3) suggest that this compound is a 3,4′ disubstituted quercetin derivative. Methylation followed by acid hydrolysis gave a product with the UV properties of quercetin 5,7,3′-tri-*O*-methyl ether. Partial hydrolysis gave four products, which were eluted and analysed for their UV and electrophoretic properties (Table 1). The first, R_f 0.00 was identified as quercetin, the second at R_f 0.11 as quercetin 4′-sulphate (λ_{max} in MeOH, nm: 251, 264 sh and 365; +NaOMe: 276, 407 with decrease in intensity; +NaOAc: 276 and 391; +$AlCl_3$ + HCl: 262 and 403), the third with R_f 0.37 as quercetin 3-sulphate and the last, R_f 0.82 was unchanged 3,4′-disulphate.

Table 1. Chromatographic and electrophoretic properties of the new flavonol sulphates, their partial hydrolysis products and other quercetin sulphates

Flavonol	R_f values (× 100) H_2O	BAW	15% HOAc	Electrophoretic mobility*
Isorhamnetin 3-sulphate	43	47	40	1.17
Quercetin 3-sulphate	37	39	35	1.00
Quercetin 4′-sulphate	11	51	17	0.39
Quercetin 3,4′-disulphate	82	20	79	4.80
Quercetin 3,7,4′-trisulphate	90	05	87	7.0
Quercetin 3-acetyl-7,3′,4′-trisulphate†	88	14	86	7.1
Quercetin 3,7,3′,4′-tetra-sulphate†	92	05	91	8.2

* Relative to quercetin 3-sulphate, run at pH 2.2 (formic acid–acetic acid) for 5 hr at 10 V/cm.

† Isolated from *Flaveria bidentis* [1, 2].

Compound **II**. Acid hydrolysis yielded quercetin and sulphate only. UV λ_{max} (nm) in EtOH–H_2O (1:1): 270 and 322; +NaOMe: 276, 369 with decrease in intensity; +NaOAc; 270 and 361; +$AlCl_3$ + HCl: 391, 342, 300 sh and 278. Partial hydrolysis gave 5 products, identified by UV data, chromatographic and electrophoretic properties as: (1) quercetin; (2) quercetin 4′-sulphate; (3) quercetin 3-sulphate; (4) compound **I** and (5) unchanged compound **II**. There was an insufficient amount of the compound for IR, NMR and MS studies, but the UV