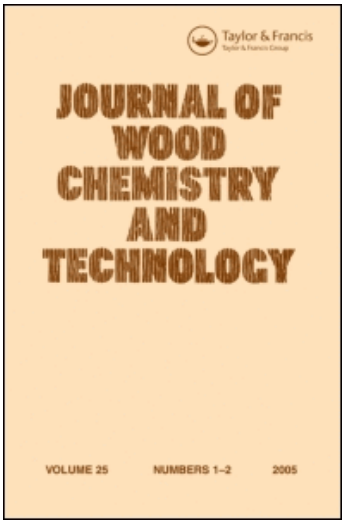

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ANALYSIS OF A HYDROXYSTILBENE GLUCOSIDE IN SEVERAL SOFTWOOD BARKS

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ABSTRACT

A method has been developed for determination of isorhapontin in softwood barks. The barks are extracted with aqueous acetone, the dried extracts re-extracted with butan-2-one, and the final extract analysed by liquid chromatography. Of seven softwood barks from five genera, only Engelmann spruce, with 13%, contained more than 1% of the stilbene glucoside.

Isorhapontin (1) has been reported by several groups of workers as a component of bark of the spruce genus. Hergert[1] surveyed the tannins and polyphenols of several softwoods and their barks by paper chromatography of extracts and noted that "several species contained stilbenes" and that "these compounds appear to be characteristic for a given species". The numbers of unidentified stilbenes cited by Hergert as possibly present in barks of twelve softwoods were greatest for Engelmann and sitka spruce (Picea sitchensis). Subsequently, Purves and co-workers[2] determined the chemical structure of a sample of (1) which Harwood[3] had isolated in 0.24% yield from the bark of white spruce. The latter reference is still the only report in the literature which cites an actual yield of (1) from a softwood bark, but Purves and co-workers[3] made

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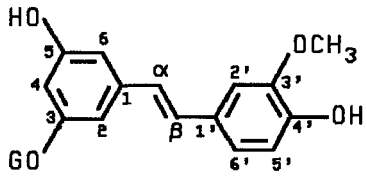
some general comments on presence of stilbenes in Picea sp. Later, Manners and Swan[4], using paper chromatography and UV spectrometry, identified (1), together with astringin (2) and isorhapontigenin (3) in barks of Picea Engelmannii, glauca, mariana, rubens and sitchensis, with a fourth stilbene, astringenin (4), primarily detected in the Engelmann, glauca and sitchensis species.



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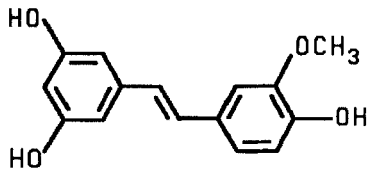

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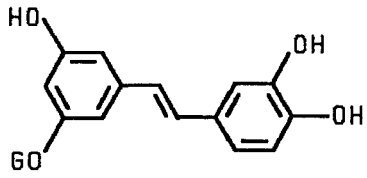

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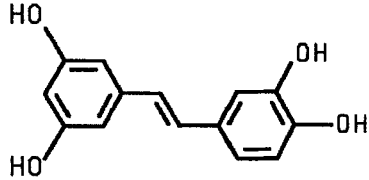

4

G = β-D-glucopyranosyl

Again, no yields of the stilbenes were reported but the authors concluded that "the taxonomic significance of the stilbenes identified in this investigation is negligible since all species displayed relatively the same pattern of stilbenes in their barks; furthermore, production is dependent upon the time of year". In a later study, Pearson et al.[5] advocated the use of (1) and (2) as "chemical markers which could be used to distinguish between Colorado blue spruce (Picea pungens Engelm.) and Engelmann spruce". Their paper described the isolation and structure elucidation of (1)

and (2) from the latter bark, but mentioned the blue spruce only in the above quotation.

In the course of our general studies of ion exchange properties and thermochemistry of softwood barks, we had noted what appeared to be a remarkably high content of stilbenes in bark from Engelmann spruce. Accordingly, and in view of the above qualitative descriptions of stilbenes in softwood barks, and the almost complete lack of quantitative data, we have devised a simple method for analysis of (1) in softwood barks. The method is of relatively low sensitivity but may be useful for certain chemotaxonomic purposes.

The method requires the extraction of the ground bark by soaking with aqueous acetone for 48 hours at room temperature. We have confirmed that these conditions extract all of the isorhapontin as judged by the fact that further extraction with the same solvent gave no increased yield of (1). The extraction at room temperature also minimized extraction of other components. The extract is evaporated to dryness and the residue extracted with butan-2-one at room temperature, which extracts all of the isorhapontin and leaves many other compounds undissolved, thus simplifying the subsequent chromatography. After addition of an internal standard, (1) is determined by liquid chromatography. This simple procedure will detect 0.5% of (1) in dry bark ± 0.1%. For barks with lower isorhapontin contents there is often a problem with interference in the chromatography from other extractants. The method could obviously be made much more sensitive by use of a UV absorption detector in the liquid chromatography. However, we do not recommend this approach because of the many other UV-absorbing extractives which are likely to be present.

Results are shown in Table 1. Of the barks studied, the Engelmann spruce has an exceptionally high isorhapontin content of 13%, at least an order of magnitude greater than any of the other six species, including white spruce. These results are in complete contrast with the qualitative observations of Manners and Swan[4], who found two different stilbenes and their glucosides in five different Picea species and concluded that the stilbenes had no

TABLE 1

Yield of Isorhapontin from Barks

Bark	Yield (% dry wt.)
Sub-alpine fir (Abies lasiocarpa)	<0.5
Western Larch (Larix occidentalis)	<1
Ponderosa pine (Pinus ponderosa)	<0.5
Douglas-fir (Pseudotsoga menziesii)	<0.5
Lodgepole pine (Pinus contorta)	<0.5
White spruce (Picea glauca)	<0.5
Engelmann spruce (Picea engelmannii)	13.1

taxonomic significance in this genus. Our results now show that Picea Engelmannii is dramatically different from Picea glauca in isorhapontin content, although the earlier work suggested that they were generally similar. All of our samples were collected at the same time of year (late summer) because of the comment (without evidence) by Manners and Swan[4] that "stilbene production is dependent on time of year". Obviously, our procedure needs to be applied more widely to softwood barks in general and especially to the Picea genus and we propose to extend our studies in this direction in the future. The procedure should be useful in studies of hybridization, especially with Engelmannii and glauca, which interbreed readily. The content of isorhapontin, which may have useful biological activity, is so high in the bark of Engelmann spruce as to suggest a possible commercial exploitation of this "waste" product.

For calibration of the liquid chromatographic analysis, isorhapontin was purified by chromatography on silica gel. The ^{13}C NMR of the resultant product and of its peracetate are shown in Table 2 and compared with previously published spectra reported by Talvitie and Hamunen[6] for (1) and its peracetate from bark of Picea

TABLE 2

Comparison of ^{13}C NMR Data for Isorhapontin and its Acetate.

Carbon Atom		Shift p.p.m. (internal TMS)			
		This work		Reference[6]	
		free phenol[a]	acetate[b]	free phenol[a]	acetate[b]
glucose	C_1	102.0	98.8	103.7	98.6
	C_2	74.7	71.1	74.6	70.9
	C_3	78.0	72.1	77.9	71.8
	C_4	71.4	68.4	71.2	68.2
	C_5	77.8	72.7	77.6	72.5
	C_6	62.7	62.1	62.6	61.8
Aglycone	C_1	147.6	139.7	147.4	139.5
	C_2	108.1	110.3	108.0	110.1
	C_3	160.2	157.5	160.0	157.3
	C_4	106.5	109.5	106.3	109.3
	C_5	159.4	151.7	159.1	151.4
	C_6	117.9	112.7	117.3	112.5
	C_1'	130.3	135.7	130.2	135.4
	C_2'	110.2	114.6	110.0	114.3
	C_3'	148.6	151.3	148.4	151.0
	C_4'	140.8	139.7	140.6	139.5
	C_5'	115.9	123.0	115.8	122.8
	C_6'	121.3	119.4	121.1	119.2
	C_α	126.7	127.5	126.4	127.3
	C_β	129.8	129.9	129.8	129.6
	OCH_3	56.2	55.9	56.1	55.7

[a]solvent d_6 acetone

[b]solvent $CDCl_3$

abies. The only significant difference is in the value of the C_1 glucose shift for (1). In this respect we note that our value of 102.0 is similar to the average value of 101.5 for the corresponding carbons in catechin 5-O- and ent-catechin 7-O-β-D-glucopyranosides[8]. The latter spectra were determined in DMSO-d_6 and D_2O and in this solvent, we found that (1) gave 101.7 ppm for the carbon atom in question.

EXPERIMENTAL

Bark samples were collected and milled as described earlier.[7] Samples (5 g) of the ground bark were covered with aqueous acetone (20% water, 500 mL), degassed with stirring under vacuum and kept for 48 hr in the dark at 2°C. Extracts were filtered and stored at 2°C in the dark.

An aliquot portion (50 mL) of each extract was taken to dryness by rotary evaporation followed by vacuum drying at 40°C. A 1% solution of 2-methoxyethanol (as an internal standard) in butan-2-one (10 mL) was added to the residue and ultrasonicated for 10 min. The resultant solution was filtered before analysis by HPLC.

The HPLC eluent was butan-2-one at a flow rate of 2.8 mL/min using a Waters Porasil radial compression column at room temperature and detection by differential refractometry. 2-Methoxyethanol eluted at 2.6 min and isorhapontin at 5.0 min. The response factor of isorhapontin with respect to 2-methoxyethanol was determined using isorhapontin isolated from Engelmann spruce bark. The isorhapontin was purified by chromatography on a silica gel column with tetrahydrofuran and gave the ^{13}C NMR spectrum shown in Table 2.

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REFERENCES

1 H.L. Hergert, For. Prod. J., 610-617 (1960).

2 D.H. Andrews, J.C. Hoffman, C.B. Purves, H.H. Quon and E.P. Swan, Can. J. Chem., 46, 2525-2529 (1968).

3 V.D. Harwood, Ph.D. thesis, McGill Univ., Montreal, Canada (1949).

4 G.D. Manners and E.P Swan, Phytochem., 10, 607-610 (1971).

5 T.W. Pearson, G.S. Kriz and R.J. Taylor, Wood Sci., 10, 93-98 (1977).

6 A. Talvitie and A. Hamunen, Finn. Chem. Lett., 60-62 (1982).

7 T.L. Lowary and G.N. Richards, J. Wood Chem. and Tech., 8, 393-412 (1988).

8 G. Nonaka, E. Ezaki, K. Hayashi and I. Nishioka, Phytochem., 22, 1659 (1983).