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A SIMPLE METHOD FOR THE ISOLATION OF SALICORTIN, TREMULACIN, AND TREMULOIDEN FROM QUAKING ASPEN (*POPULUS TREMULOIDES*)

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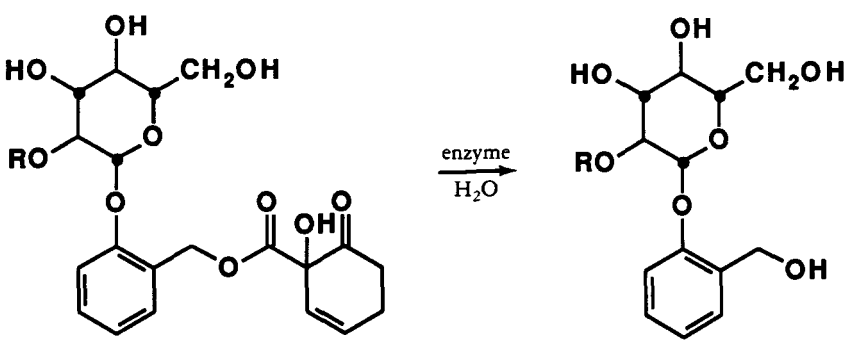
Phenolic glycosides are a widespread and diverse class of compounds found in many plants. Over the past 20 years, their occurrence has elicited interest in the fields of chemotaxonomy (1,2), medicinal chemistry (3–5), and chemical ecology (6–11). Unfortunately, these studies have often been hindered by the unavailability of large quantities of these same compounds.

Recently Lindroth *et al.* (12) reported a method for isolating salicortin [**1**], salicin [**2**], tremulacin [**3**], and tremuloiden [**4**] (Figure 1) from quaking aspen (*Populus tremuloides*) foliage. In this note we report a dramatically improved method for isolating these substances from quaking aspen. The advantages of our method are: (a) aspen internodes, rather than leaves, are used as the source of phenol glycosides because internodes have a predictable phenol glycoside content, while leaf phenol glycoside content fluctuates dramatically during the growing season (13,14), (b) the presence or absence of a simple pretreatment of internode extracts directs which glycosides are isolated, and (c) chromatographic separations of these phenol glycoside mixtures are trivial compared to separations of the mixtures extracted from leaves.

Quaking aspen internodes contain salicortin [**1**] and tremulacin [**3**] as the only major Me_2CO-soluble secondary metabolites (14). Tremuloiden [**4**] and pigments, which are somewhat difficult to separate from tremulacin [**3**], are essentially absent from the internodes, making the isolation of **3** trivial. Similarly, the absence of salicin [**2**] allows easier recovery of **1**. We have isolated 2.2 g of **3** and 4.8 g of **1** from internodes in a single chromatographic run. While ^1H- and ^{13}C-nmr spectral analyses indicate that the isolated glycosides are of high purity, a final purification can be obtained after a single run by either re-



1 R=H
3 R=benzoate

2 R=H
4 R=benzoate

FIGURE 1. Structures of the four major phenol glycosides of *Populus tremuloides*: salicortin [**1**], salicin [**2**], tremulacin [**3**], and tremuloiden [**4**].

crystallization or by additional chromatography using a different solid support (see Experimental).

Tremuloiden [**4**] can be obtained from aspen internodes using a preparation from aspen foliage that converts tremulacin [**3**] and salicortin [**1**] into tremuloiden [**4**] and salicin [**2**], respectively. Internodes are simply freeze-dried, ground, and suspended in H_2O containing a small amount of crushed leaves. When all of **3** has been converted to **4** (monitored by tlc), the suspension is worked up and again chromatographed. In this case the only major component to be separated from tremuloiden is salicin, making the separation facile. In one chromatographic run followed by recrystallization from Me_2CO, 2.8 g of pure (as determined from nmr analyses) **4** was isolated.

The empirical control over isolation of tremulacin/tremuloiden and salicortin/salicin is reminiscent of our earlier report (15) of the isolation of trichocarpin or trichocarpigenin (benzyl gentisate) from balsam poplar (*Populus balsamifera*) depending upon the extraction procedure. While all the factors which influence the isolation of these substances are not known, we suspect that enzymatic transformations play important roles in both of these systems (14–16).

EXPERIMENTAL

PLANT MATERIAL.—The plants (*Populus tremuloides* Michx.) were collected at the University of Alaska, Fairbanks, and were identified by Dr. John Bryant, Institute of Arctic Biology, University of Alaska, Fairbanks. Voucher specimens are deposited at the University of Alaska herbarium (ALA; Clausen 1).

ISOLATION OF TREMULACIN [**3**] AND SALICORTIN [**1**].—Freeze-dried internodes of *Populus tremuloides* second-year growth, 570 g) were ground and extracted overnight in 2 liters of Me_2CO. After filtration and removal of the Me_2CO, the extract (23 g) was mixed with about 5 g of Si gel and loaded on top of a flash chromatography column (1000 ml capacity) packed with Si gel (40 μm). Elution using standard procedures (17) with $CHCl_3$-MeOH (85:15) resulted in a clean separation of **3** from **1**. Tremulacin was easily purified by recrystallization (EtOAc/$CH_3C_6H_5$) to yield chromatographically pure material. Verification of purity was accomplished by comparing nmr spectra with the ^{1}H- and ^{13}C-nmr spectra reported for **3** (12) and **1** (12,18). For further purification of **1**, chromatography on reversed-phase support (see below) was preferred over recrystallization (absolute EtOH) (19). Final yields of **3** and **1** were 2.2 g (0.4%) and 4.8 g (0.8%), respectively. Alternatively, flash chromatography using reversed-phase support (C-18, 40 μm) and H_2O-Me_2CO (58:42) eluent can be used to achieve the above separations.

ISOLATION OF TREMULOIDEN [**4**].—Dried aspen internodes (second-year growth, 300 g) were ground, and H_2O was added to cover the plant material. About ten fresh aspen leaves were crushed with a mortar and pestle with the aid of sand and H_2O. The crushed leaves along with the liquor were transferred to the beaker and periodically stirred. The progress of the conversion of **3** to **4** was monitored using tlc [Si gel, $CHCl_3$-MeOH (80:20)] of an Me_2CO extract of subsampled plant material. The R_fs of **3** and **4** are about 0.59 and 0.51, respectively, although the exact values depend on the grade of MeOH used. When the conversion was complete (about 2–4 h), an equal volume of MeOH was added. After filtration, removal of the MeOH, and lyophilization, **4** was isolated by flash chromatography as described above for **3** and **1**. Recrystallization from Me_2CO yielded 2.8 g (0.9%) of pure **4**, which gave ^{1}H- and ^{13}C-nmr spectra virtually identical to those reported in the literature (12,18).

In the above procedure, it is important to add the MeOH when the reaction is completed or the yield of **4** will be compromised due to its further hydrolyses.

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LITERATURE CITED

1. G.W. Argus and J.W. Steele, *Syst. Bot.*, **4**, 103 (1979).
2. R. Julkunen-Tiitto, *J. Chromatogr.*, **324**, 129 (1985).
3. Y. Pei, X. Li, S. Wang, S. Su, and T. Zhu, *Zhongcaoyao*, **14**, 433 (1983).
4. B. Meier and O. Sticher, *Pharm. Ind.*, **48**, 87 (1986).
5. I.A. Pearl, *Tappi*, **52**, 428 (1961).
6. E. Helle, R. Jullkunen-Tiitto, A. Lavola, and J. Tahvanainen, *Oecologia (Berlin)*, **65**, 319 (1985).
7. M. Rowell-Rahier and J.M. Pasteels, Proceedings of the 5th International Sym-

posium of Insect-Plant Relationships, Wageningen, Pudoc, Wageningen, 1982, pp. 73–79.
8. R. Lindroth, J.M. Scriber, and M.T.S. Hsia, *Oecologia (Berlin)*, **70**, 13 (1986).
9. M. Rowell-Rahier, P.H. Soetens, and J.M. Pasteels, in: "Insects—Plants." Ed. by V. Labeyrie, G. Fabres, and D. Lachaise, Dr. W. Junk, Dordrecht, 1987, pp. 91–95.
10. W.R.N. Edwards, *N.Z. J. Sci.*, **21**, 103 (1978).
11. T. Palo, *J. Chem. Ecol.*, **10**, 449 (1984).
12. R. Lindroth, M.T.S. Hsia, and J.M. Scriber, *Biochem. Syst. Ecol.*, **15**, 677 (1987).
13. R.L. Lindroth, M.T.S. Hsia, and J.M. Scriber, *Biochem. Syst. Ecol.*, **15**, 681 (1987).
14. T.P. Clausen, J.P. Bryant, P.B. Reichardt, and R.A. Werner, in: "Phytochemical Induction by Herbivores." Ed. by M. Raupp and D. Tallemy, John Wiley & Sons, New York, in press.
15. B.R. Mattes, T.P. Clausen, and P.B. Reichardt, *Phytochemistry*, **26**, 1361 (1986).
16. R.L. Lindroth and M.S. Pajutee, *Oecologia (Berlin)*, **74**, 144 (1987).
17. W.C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
18. R.A. Dommisse, L.V. Hoof, and A.J. Vlietinck, *Phytochemistry*, **25**, 1201 (1986).
19. I.A. Pearl and S.F. Darling, *Phytochemistry*, **10**, 3161 (1971).

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