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PURPUREIN, A NEW GLUCOSIDE FROM THE BARK OF *SALIX PURPUREA**

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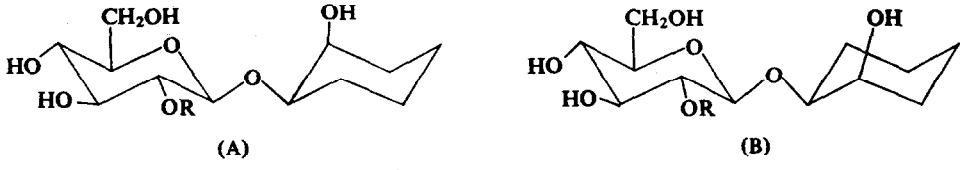
Abstract—A new glucoside, purpurein, was isolated from fresh June bark of *Salix purpurea*. Alkaline and enzymatic hydrolysis and mass spectrometry demonstrated its structure to be a *p*-coumaric acid ester of a diastereoisomer of grandidentin with *p*-coumaroyl substitution on the glucose moiety. NMR spectrometry suggested that substitution was on the 2-*O*-glucose position.

INTRODUCTION

IN A RECENT study on the hot-water extractives of the fresh June bark of *Salix purpurea* reeds,[1] we reported the isolation by polyamide chromatography of a new glucoside "purpurein". The structure of purpurein has been established as a *p*-coumaroyl ester of *cis*-2-hydroxycyclohexyl-β-D-glucopyranoside and mass spectrometry and NMR spectrometry suggest the formulation *cis*-2-hydroxycyclohexyl-2-*O*-*p*-coumaroyl-β-D-glucopyranoside (I, A or B), a diastereoisomer of grandidentatin.[2]



(A) (B)

(I) R = -*p*-coumaroyl
(II) R = H

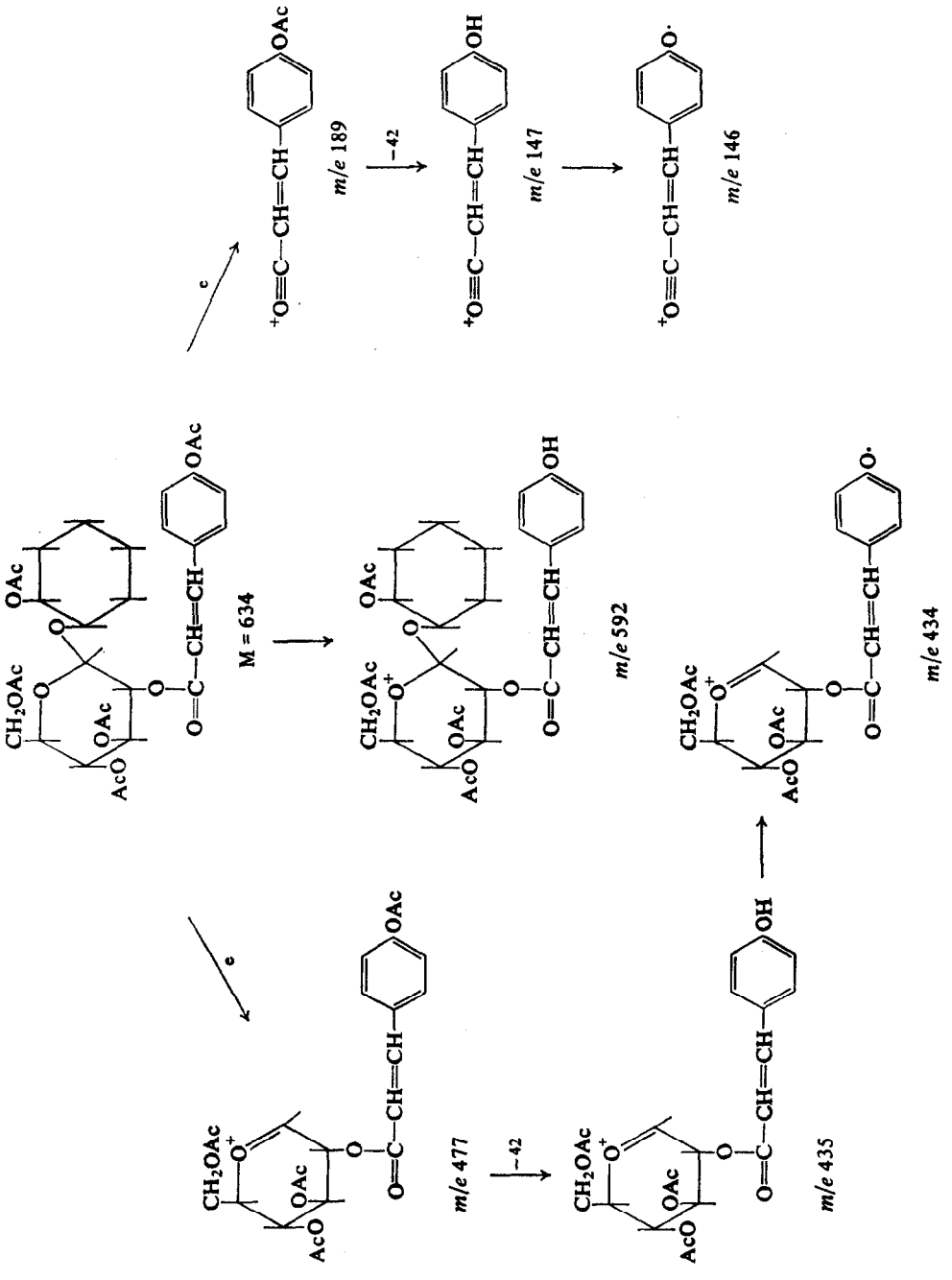
RESULTS

The structure I (A or B) was established in the following manner. Alkaline hydrolysis of purpurein yielded *p*-coumaric acid and a glucoside (II, A or B) which, upon hydrolysis with β-glucosidase, yielded glucose and *cis*-1,2-cyclohexanediol. The de-*p*-coumaroylated purpurein (II, A or B) was acetylated and analysis and i.r. and mass spectra of the acetate III demonstrated that II (A or B) was a diastereoisomer of grandidentin.[2]

Purpurein was acetylated and the resulting acetate IV was subjected to analysis and mass spectrometry. The mass spectrum of IV is essentially identical with that of grandidentatin pentaacetate confirming the molecular weight and the fact that the *p*-coumaroyl substitution is on the glucose moiety.[3] The major fragmentation pattern of purpurein pentaacetate (IV) is pictured in Scheme I which accounts for the important peaks in the mass spectrum.

* Part XXII in the series "Studies on the Barks of the Family Salicaceae".

[1] I. A. PEARL and S. F. DARLING, *Phytochem.* **8**, 2393 (1969).
[2] I. A. PEARL and S. F. DARLING, *J. Org. Chem.* **27**, 1806 (1962).
[3] I. A. PEARL and S. F. DARLING, *Phytochem.* **7**, 831 (1968).


e
M = 634
m/e 189
-42
m/e 147
m/e 146
m/e 592
m/e 434
m/e 477
-42
m/e 435

An attempt to locate the position of *p*-coumaroyl substitution on the glucose moiety by methylation followed by saponification and acid hydrolysis, so successfully employed in the past for determining the structure of tremuloidin[4] and grandidentatin,[2] failed because the employment of either the traditional Purdie method or the Walker, Gee and McCready[5] modification of this methylation procedure resulted in the production of both 2,3,4-tri-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose proving that migration of the *p*-coumaroyl group took place during the methylation procedure.

The NMR spectrum of purpurein determined under the same conditions employed previously for grandidentatin and grandidentoside[6] contained the same peaks exhibited by the latter two glucosides in the 5·0–6·0 ppm τ region corresponding with shifts of the H-1 and H-2 protons identical with those of the known C-2 esterified compounds.[6] It is improbable that C-3 or C-4 esterification would cause the 0·5 ppm downfield shift of H-1 noted here, and it has been demonstrated that C-6 esterification caused downfield shifts of two protons.[6] Thus, although not established unequivocally, NMR spectrometry suggests substitution at the C-2 position on the glucose moiety and the formula I (A or B) for purpurein. Further work is required to define the absolute stereochemistry of grandidentatin or purpurein as IA or IB and similarly of grandidentin and its diastereoisomer as IIA or IIB.

EXPERIMENTAL*

Preparation of Purpurein

In the polyamide chromatogram of *S. purpurea* bark ethyl acetate Extract A described in a previous paper,[1] concentration to small volumes under reduced pressure of eluate fractions 46 to 58 yielded a total of 0·755 g of colorless prisms which were recrystallized from water containing a little ethanol to give colorless needles of purpurein hydrate melting at 112–113°, and having a specific rotation in 80 per cent acetone of $[\alpha]_D^{25}$ −39·3° (*c.* 2·2). Its i.r. spectrum (KBr disc) contained bands at 3475, 2915, 1695, 1630, 1605, 1515, 1440, 1365, 1330, 1260, 1195, 1165, 1075, 1062, 1031, 912, 888, 859 and 831 cm^{-1} and was essentially similar to that of grandidentatin.[2] (Found: C, 54·30, 54·51; H, 7·07, 7·09. $C_{21}H_{28}O_9.2H_2O$ required: C, 54·77; H, 7·00%.)

The NMR spectrum (60 MHz) was determined in methyl sulfoxide-d_6 containing deuterium oxide with tetramethylsilane as an internal standard. The spectrum exhibited in the important 5·0–6·0 ppm τ region[6] an H-2 triplet centered at 5·28 ppm and a partially overlapping H-1 doublet centred at 5·52 ppm, shifts almost identical with those obtained with grandidentatin and grandidentoside.[6]

Acetylation with acetic anhydride and pyridine and recrystallization from dilute ethanol yielded purpurein pentaacetate (IV), m.p. 140–141°, $[\alpha]_D^{25}$ −34·4° (*c.* 2·91 in $CHCl_3$). Its i.r. spectrum (KBr disc) was substantially similar to that of grandidentatin pentaacetate[2] and contained bands at 3450, 2940, 1755, 1642, 1605, 1510, 1430, 1370, 1315, 1210, 1150, 1090, 1035, 986, 952, 918, and 846 cm^{-1}. The mass spectrum contained the following major and important *m/e* peaks with relative *m/e* intensity for each peak noted in parentheses: 43 (100), 81 (24·9), 97 (7·9), 98 (4·8), 99 (16·4), 141 (18·8), 146 (16·4), 147 (92·0), 148 (8·5), 164 (13·3), 169 (8·5), 189 (54·5), 190 (6·1), 228 (3·6), 368 (2·4), 389 (9·1), 390 (9·0), 434 (5·9), 435 (2·3), 477 (2·9), 552·8 [this is a metastable peak corresponding with the loss of acetyl as ketene from the molecular ion], 592 (2·1) and M^+ 634 (0·05). Except for actual relative intensities, this mass spectrum is practically identical with that of grandidentatin pentaacetate whose mass spectral fragmentation was discussed in a previous paper.[3] (Found: C, 58·50, H, 5·97, mol. wt. by mass spectrometry, 634. $C_{31}H_{38}O_{14}$ required: C, 58·67; H, 6·04; mol. wt. 634.)

Alkaline Hydrolysis of Purpurein

Purpurein (96·0 mg) was saponified with $Ba(OH)_2$ solution in the same manner reported for grandidentatin[2] and the ether extract of the acidified mixture yielded 35·2 mg (100 per cent) of the hydrate of *trans-p*-coumaric acid, identified by mixed m.p. and identity of i.r. absorption spectrum with that of authentic material. The aqueous raffinate yielded de-*p*-coumaroylated purpurein (II A or B) as 61·5 mg of colorless sirup which would not crystallize.

* All m.p.s are uncorrected. Analyses were performed by Micro-Tech Laboratories, Skokie, Illinois, and mass spectra were determined by Morgan-Schaffer Corp., Montreal, Quebec, Canada. I.r. and NMR spectra were determined at The Institute of Paper Chemistry by Lowell Sell and R. L. Erickson, respectively.

[4] I. A. PEARL and S. F. DARLING, *J. Org. Chem.* **24**, 731 (1959).
[5] H. G. WALKER, M. GEE and R. M. MCCREADY, *J. Org. Chem.* **27**, 2100 (1962).
[6] R. L. ERICKSON, I. A. PEARL and S. F. DARLING, *Phytochem.*, **9**, 857 (1970).

The sirup II (A or B) was acetylated with acetic anhydride and pyridine and the product was recrystallized from dilute ethanol to yield de-*p*-coumaroylated purpurein pentaacetate (III) as colorless silky needles, m.p. 112–113°, $[\alpha]_D^{25}$ +8·25° (*c*. 1·7 in $CHCl_3$). Its i.r. spectrum contains bands at 2945, 2865, 1739, 1428, 1378, 1368, 1322, 1251, 1225, 1170, 1131, 1120, 1090, 1066, 1040, 978, 956, 942, 931, 836, 820, 709, 675, 645, 630, 606, 598, 561, 500, 479, 457, 435, 414, 392, 364, 345, 336, 330, 323, 318, 304, 289, and 284 cm^{-1} and was essentially similar to that of grandidentin pentaacetate determined on the same instrument. The mass spectrum contained the following major and important *m/e* peaks: 43 (100), 81 (28), 97 (6·4), 98 (19·8), 99 (21·7), 103 (8·3), 109 (15·1), 112 (8·7), 115 (17·2), 127 (6·4), 140 (10·8), 141 (21·7), 145 (10·0), 157 (16·8), 169 (17·3), 200 (8·3), 242 (6·7), 271 (0·7), 331 (2·1), and M^+ 488 (0·0004). This spectrum is identical with that of grandidentin pentaacetate. (Found: C, 54·18, 54·35; H, 6·70, 6·78; mol. wt. by mass spectroscopy, 488. $C_{22}H_{32}O_{12}$ required: C, 54·09; H, 6·60; mol. wt. 488.)

Enzymatic Hydrolysis of De-p-coumaroylated Purpurein

The de-*p*-coumaroylated product II (A or B) was hydrolyzed in acetate buffer with β-glucosidase as described previously.[2] The ether extract of the reaction mixture yielded colorless plates of *cis*-1,2-cyclohexanediol melting at 94–95°, identified by mixed m.p. and identity of i.r. spectrum with that of authentic material.[2] Glucose was identified in the aqueous raffinate by paper chromatography and reaction with *p*-nitroaniline to form *N*-(*p*-nitrophenyl)-α-D-glucopyranosylamine, identified by mixed m.p. with authentic compound.[7]

Methylation of Purpurein

Purpurein was methylated in one step with methyl iodide and silver oxide in dimethylformamide by the Walker, Gee and McCready modification employed earlier for salireposide.[8] The methylated product was hydrolyzed with methanolic hydrogen chloride, and the hydrolyzate was examined by paper chromatography as described earlier.[4] Spots for 2,3,4-tri-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose were obtained, proving that the *p*-coumaroyl group had migrated and partially hydrolyzed during the methylation process. *p*-Methoxy-cinnamic acid was isolated from the methylation mixture. The traditional Purdie procedure, which was employed so successfully with grandidentatin,[2] gave results practically identical with those obtained by use of the rapid modification.

Mass Spectra

Mass spectra were made on a double-focusing Hitachi RMU-6D instrument by direct introduction of the samples with a probe in the ionizing beam.

[7] F. WEYGAND, W. PARKOW and P. KUHNER, *Chem. Ber.* **84**, 594 (1951).
[8] I. A. PEARL and S. F. DARLING, *Phytochem.* **7**, 821 (1968).