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DODECAACETYLPRODELPHINIDIN B3 FROM THE DRIED LEAVES OF *ZIZIPHUS SPINA-CHRISTI*

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Key Word Index—*Ziziphus spina-christi*; Rhamnaceae; dried leaves; dodecaacetylprodelphinidin B3; ^{13}C NMR.

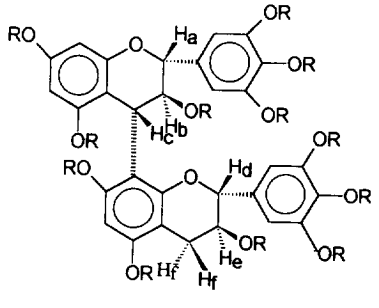
Abstract—The ethanolic extract (10% of the dried leaves) of *Ziziphus spina-christi* was acetylated leading to the isolation of a crystalline dodecaacetylprodelphinidin B3, which has not been described in the literature. Its structure was determined on the basis of its ^{1}H and ^{13}C NMR spectra. The main components of the extract are glucose (4.4%), sucrose (21%), dimers, oligomers and polymers of prodelphinidins (16%), betulinic acid (1.7%), sitosterol β-D-glucoside (2%), (+)-gallocatechin (1.7%) and (−)-epigallocatechin (0.9%). The structures of the crystalline peracetates were proven by comparison with the physical properties described in the literature. Proanthocyanidins and betulinic acid seem to be chemotaxonomically characteristic of *Ziziphus* species.

INTRODUCTION

The economic importance of the Rhamnaceae is insignificant, providing some lumber and a small number of ornamental shrubs. Up to now, from a large number of *Ziziphus* species only *Z. jujuba* and *Z. maritiana* have been chemically investigated [1], due to their cultivation as fruit trees. They contain large quantities of tannins and were therefore used locally for tanning. However, a detailed analysis of the chemical nature of the tannins has not been reported [1]. The dried and finely ground leaves of *Z. spina-christi* are used as an additive in some natural hair dying products, whereby special colour shades can be obtained. Since the material has not been studied before, it was of interest, for ecological and chemotaxonomical reasons, to determine the principle constituents of this plant.

RESULTS AND DISCUSSION

After briefly heating the leaves of the dried plant in dimethylformamide containing a few drops of concentrated hydrochloric acid, an intense red solution resulted. It was chromatographically determined that the solution contained delphinidinchloride. For this reason the plant must contain (*vide infra*) prodelphinidins and was worked-up according to our isolation method for proanthocyanidins [2]. According to this method the ethanolic extract is crudely fractionated on a flash Perlon column into three fractions (A = 72%, B = 12%, C = 4%). The mixtures in the three fractions were acetylated since the acetylated natural substances separate better on flash silica columns and generally crystallize well. From the acetylated fraction A acetylbetulinic acid, sitosterol-tetraacetyl-β-D-glucoside, pentaacetylglucose and octaacetyl-sucrose were isolated. From acetylated fraction B hexaacetyl (+)-gallocatechin and hexaacetyl (−)-epigallocatechin were isolated. Finally, the acetylated fraction C contained four peracetylated prodelphinidins, from which the dodecaacetylprodelphinidin B3 (**1a**) could be isolated in crystalline form. Up to now crystalline peracetylated prodelphinidins have not been described in the literature. The constitution and configuration of **1a** could be deduced by direct comparison of the four diastereomeric decaacetylprocyanidins with those described in the literature [3]. The ^{1}H NMR spectrum of **1a** differs from the decaacetylprocyanidin B3 only in the region of the



1 R = H

1a R = $COCH_3$

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Table 1. ^{13}C-chemical shifts of dodecaacetylprodelphinidin (**1a**), hexaacetyl-(+)-gallocatechin (**2**) and hexaacetyl-(−)-epigallocatechin (**3**)

C	1a	2	3
2	77.5	77.3	76.4
2′	78.8	—	
3	68.1	68.1	66.4
3′	70.3	—	—
4	36.6 *t*	24.0 *s*	25.8 *s*
4′	24.8 *s*	—	—
quart.	111.3	110.1	109.5
aromat.	115.1	134.5	134.2
	116.7	135.7	135.4
	135.0	143.4	143.2
	135.1	149.3	149.6
	143.1	149.7	149.6
	143.1	154.1	154.7
	147.5		
	147.9		
	148.8		
	149.5		
	152.4		
	155.6		
tert.	18.1	107.6	108.0
aromat.	109.5	108.8	108.8
	110.2	118.8	118.9
	118.6		
	119.5		

The signals of the acetyl groups are not included in the table. They lie at δ 166–171 for COMe and at $\delta = 20$–21 for COCMe.

aromatic and acetyl protons. Signals for the aliphatic protons H_a–H_e are in the range $\delta = 4.96$–5.63 and both H_f protons are at $\delta = 2.57$–2.90, in analogy to the protons of decaacetylprocyanidin B3, with the same coupling constants.

From the above it is clear that **1a** is formed by the oxidative coupling of two molecules of (+)-gallocatechin. Comparison of the ^{13}C NMR spectrum of **1a** with that of hexaacetyl (+)-gallocatechin and hexaacetyl (−)-epigallocatechin confirm this structure.

EXPERIMENTAL

All mps are uncorr. NMR: Bruker AC-200. Optical rotation: Perkin–Elmer polarimeter 141; Uvasol solvent (Merck). TLC: precoated plates, Polygram SIL G/UV$_{254}$ (Macherey–Nagel). TLC developer: conc. H_2SO_4–formalin (9:1); development: 10 min at 120°. CC: flash columns filled with either silica gel 32–63 (ICN-Biochemicals) or Perlon powder as in ref. [2].

Extraction. A portion (500 g) of the ground leaves of *Ziziphus spina-christi* was extracted 3 × with 2 l of 95% EtOH (24 hr) at room temp. The alcoholic extracts were sepd from the leaves by vacuum filtration through a cotton pad at 50°C bath temp. and evapd to *ca* 400 ml. The extract was allowed to stand overnight, after which 50 g of Celite was added and sepd by gravity filtration. A clear green soln results which contains *ca* 50 g of dried substances (10% based on the dried leaves).

Crude separation of the extract via flash Perlon CC. Portions (100 ml) of the extract were loaded on to a flash Perlon column (30 × 6 cm) and eluted with 95% EtOH. Each of the frs consisted of 500 ml. After elution with *ca* 2 l of 95% EtOH the eluent was practically colourless and the column was eluted with 1 l EtOH–DMF (8:2). The individual frs were examined via TLC ($CHCl_3$–MeOH–2 N HOAc, 70:30:6). The frs containing the same products were combined and concd on a rotary evaporator, which resulted in 3 frs. The reported yields are based on the original 400 ml extract containing 50 g of dry substance.

Fr. A: 36 g (72%), fr. B: 6 g (12%), fr. C: EtOH–DMF soln 2 g (4%). Polymeric tannins 6 g (12%) which remained on the Perlon powder were eluted with 100% DMF, but were not examined further.

Acetylation of the frs. The residues of frs A–C were dried over P_4O_{10} and each was dissolved in dry pyridine–Ac_2O (for 1 g residue: 1.6 ml pyridine and 2 ml Ac_2O) and stirred for 12 hr at room temp. The resulting soln was poured on to ice–H_2O and the pptd products were collected by vacuum filtration, dissolved in EtOH and heated to boiling for a short time after the addition of activated charcoal. After gravity filtration the soln was evapd to dryness and the solid residue was dried under vacuum over P_4O_{10}.

Separation of the acetylated fr. A. From 15 g of fr. A 16 g of acetate mixt. was isolated. This mixt. was dissolved in toluene and filtered. The resulting clear green soln was found to contain 11 compounds according to TLC (toluene–acetone 7:3), of which the 4 main components were obtained in crystalline form. The toluene soln was sepd on a flash silica gel column (30 × 6 cm, toluene–acetone gradient: 19:1 → 4:1) and the frs analysed via TLC (toluene–acetone 7:3)

Fraction A1. R_f practically running with the solvent front contained 2 green substances which were not sepd further due to insufficient material.

Fraction A2. R_f 0.8–0.9, yield = 1.2 g; contained 3 substances, 2 of which were isolated in crystalline form by dissolving the mixt. in *n*-hexane–diethyl ether (19:1) and sepn on a flash silica column (30 × 3.5 cm, *n*-hexane–diethylether gradient: 19:1 → 4:1).

3-Acetylbetulinic acid. Yield = 0.4 g; recrystallized from *n*-hexane. mp 286–289°, R_f 0.64 (*n*-hexane–diethyl ether 1:1). All physical properties agree with those in the lit. [4]. $C_{32}H_{50}O_4$ (M_r = 498.75) calcd. C 77.06, H 10.10; found C 77.27, H 10.16.

Sitosterol-tetraacetyl-β-D-glucoside. Yield = 0.6 g; recrystallized from *n*-hexane–diethyl ether; mp = 170°C; R_f 0.25 (*n*-hexane–diethyl ether 1:1]. All physical properties agree with those in the lit. [5]. $C_{43}H_{68}O_{10}$ (M_r = 745.00), calcd C 69.32, H 9.19; found C 69.23, H 9.01.

Fraction A3. R_f 0.57; yield = 2 g; recrystallized from EtOH. All physical properties were identical to those of authentic sample of pentaacetylglucose.

Fraction A4: R_f 0.46; yield = 9 g; recrystallized from EtOH. All physical properties were identical to those of authentic sample of octaacetylsucrose.

Fraction A5. R_f = 0.2–0.4; yield = 2.5 g. Contained 4 substances which have not, as yet, been further sepd.

Ca 1.2 g of a brown material remained at the origin of the silica gel column.

Separation of the acetylated fr. B. From 6 g of fr. B 6 g of the acetate mixt. was obtained which was sepd analogous to fr. A.

Fraction B1. R_f = 0.47; yield = 1.6 g; recrystallized from EtOH. Hexaacetylgallocatechin: mp = 141–142°; $[\alpha]_D$ = +31 (acetone; *c* 1). All physical properties agree with those in lit. [6]. ^{13}C NMR: Table 1.

Fraction B2. R_f = 0.54; yield = 0.8 g; crystallized from EtOH. Hexaacetylepigallocatechin: mp 191°; $[\alpha]_D$ − 14 (acetone; *c* 1). All physical properties agree with those in the lit. [6]. ^{13}C NMR: Table 1.

Separation of the acetylated fr. C. From 2 g of fr. B 2.4 g of the acetate mixt. was obtained which was sepd analogous to fr. A. Four frs were obtained and after evapn of solvent the fr. C3 was recrystallized from EtOH.

Dodecaacetylprodelphinidin (**1a**). Yield = 0.4 g; mp = 184–186°; R_f = 0.24 [toluene–acetone (7:3)]: $[\alpha]^{20}_{589}$ − 98.9; $[\alpha]^{20}_{578}$ − 103.4; $[\alpha]^{20}_{546}$ − 119.8 (acetone; *c* 1); ^{13}C NMR: Table 1. $C_{54}H_{50}O_{26}$ (M_r 1114.98); calcd. C 58.17, H 4.52; found C 58.01, H 4.49.

The other substances are diastereomeric dodecaacetylprodelphinidins which are formed under the reaction conditions with warm DMF–conc. HCl yielding delphinidin chloride, as chromatographically determined.

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