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o-(α-L-RHAMNOPYRANOSYLOXY)BENZYLAMINE AND *o*-HYDROXYBENZYLAMINE IN *RESEDA ODORATA*

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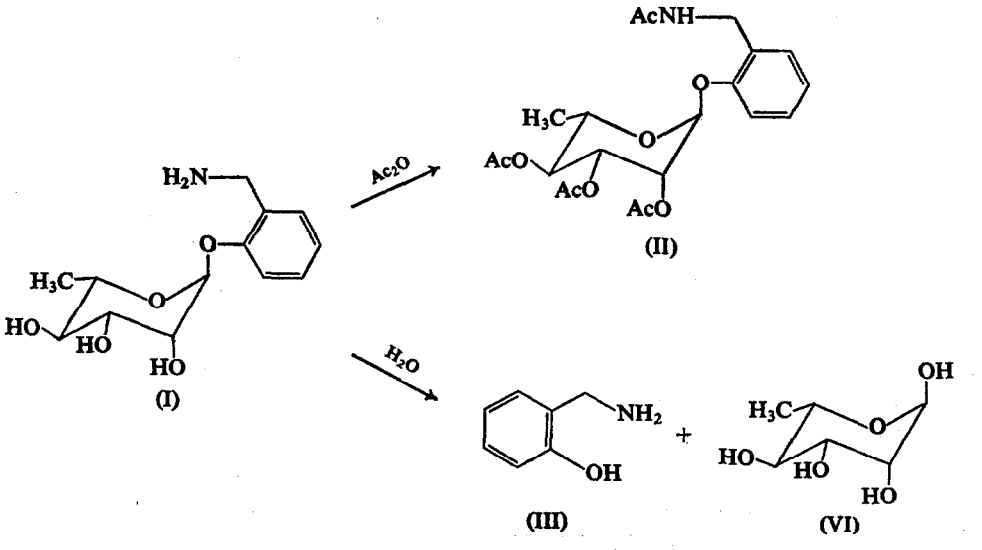
Abstract—Two new natural products, *o*-(α-L-rhamnopyranosyloxy)benzylamine and *o*-hydroxybenzylamine, have been isolated from flowers of *Reseda odorata* L. The proposed structures have been confirmed by transformation of the rhamnoside into N-[o-(*O*-triacetyl-α-L-rhamnopyranosyloxy)benzyl]acetamide and by synthesis of this amide and the two amines. A possible biogenetic relationship between the amines and glucosinolates is briefly discussed.

INTRODUCTION

IN PREVIOUS communications from this laboratory investigations on the amino acids and amines in plants containing glucosinolates have been reported (see Ref. 1, 2 and references cited therein). One result of this work was the demonstration of *p*-hydroxybenzylamine in *Sinapis alba* L. (Cruciferae).[3] During these studies and concurrent biosynthetic investigations,[4] the free amino acids in flowers of *Reseda odorata* L. (Resedaceae) were chromatographed and several unknown compounds were revealed. Two of these on paper



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[2] P. O. LARSEN, *Acta Chem. Scand.* **22**, 1369 (1968).
[3] P. O. LARSEN, *Biochim. Biophys. Acta* **107**, 134 (1965).
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with ninhydrin gave a colour reaction indicative of benzylamine derivatives;[5] their R_fs were similar to those of *p*-hydroxybenzylamine.

The two compounds have now been isolated and identified as *o*-(α-L-rhamnopyranosyloxy)-benzylamine (I) and *o*-hydroxybenzylamine (III). The structures have been determined by spectroscopic methods, by identification of the hydrolysis products, by transformation of I into the tetraacetyl derivative (II) and by comparison with the *p*-compounds corresponding to I, II, and III.[6] Final confirmation has been achieved by synthesis of I, II, and III.

RESULTS

Isolation of I and III from flowers of *Reseda odorata* L. was performed by homogenization and defatting of the plant material with CCl_4, extraction with methanol–water, fractionation on ion-exchange resins and preparative paper chromatography. Final purification of III was accomplished by sublimation. The yields were small due to decomposition during the isolation procedure. The glycosidic bond in I is labile and partial hydrolysis occurs on strongly acid ion-exchange resins in the hydrogen form. III is easily oxidized by oxygen to coloured compounds.[7]

Chromatographic investigations of the crude plant extract showed the ratio between I and III to be about 5:1. Extraction under very mild conditions (see Experimental) demonstrated the presence of both I and III, indicating that none of the compounds is an artefact produced during isolation. Acetylation of I (both natural and synthetic) was performed with acetic anhydride in pyridine at room temperature and gave a nearly quantitative yield of N-[*o*-(*O*-triacetyl-α-L-rhamnopyranosyloxy)benzyl]acetamide (II).

The NMR spectrum of I (in D_2O) showed the four aromatic protons at δ=6·9–7·4 as a nearly symmetrical pattern. The proton at C_1 in the rhamnose part of the molecule occurred mainly as a doublet at δ=5·5 (J=2 c/s) corresponding to the conformation depicted, whereas a smaller doublet at δ=5·0 (J=8 c/s) indicates the presence of a smaller amount of the conformer in which the protons at C_1 and C_2 are both axial.[8,9] The two benzylic protons and the four protons at C_2, C_3, C_4 and C_5 in the rhamnose part of the molecule appeared as a complex pattern at δ=3·2–4·2. The protons from the methyl group appeared as a doublet at δ=1·25 (J=5·5 c/s) in agreement with the literature.[9]

The NMR spectrum of II (in $CDCl_3$) showed the four acetyl groups as four singlets at δ=2·0–2·2, the amide proton in a broad band at δ=2·7 (disappearing on addition of D_2O), the three protons at C_2, C_3 and C_4 in the rhamnose part at δ=5·0–5·6 and the C_5 proton as a complex pattern at δ=4. Decoupling experiments demonstrated coupling of the C_5 proton with the protons of the methyl group appearing at δ=1.25 (J=5·5 c/s). The benzylic protons appeared as a symmetrical quartet at δ=4·5 (J=6 and 2 c/s) with the splittings caused by non-equivalence and long-range couplings. The anomeric proton and the aromatic protons appeared with the same δ values as in I.

The mass spectra of III and of *p*-hydroxybenzylamine were nearly identical, deviating only in the relative intensities of some of the peaks. The differences corresponded to those noted in

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the mass spectra of *o*- and *p*-hydroxybenzyl alcohol.[10] The intensities of the peaks in the mass spectra of I and II and of the *p*-derivative corresponding to II were strongly dependent on the inlet temperature of the mass spectrometer. The spectrum of I showed a significant peak at *m/e* 269 (M^+), peaks corresponding to peaks in the spectrum of III and a number of peaks attributable to fragmentation of the rhamnose part of the molecule. The mass spectra of II and the corresponding *p*-compound were almost identical. Both compounds gave a very weak peak at *m/e* 437 (M^+), important peaks at *m/e* 273 (the rhamnose part), *m/e* 165 (the hydroxybenzylamine moiety) and also peaks characteristic of III and of *p*-hydroxybenzylamine respectively. In addition a number of peaks (*m/e* 213, 171, 153, and 111) must be ascribed to fragmentation of the rhamnose part, in agreement with data in the literature for the mass spectrum of peracetyl-6-deoxyglucose.[11]

R_fs on paper and silica gel plates (see Tables 1 and 2) are invariably smaller for the *p*-derivatives than for the corresponding *o*-derivatives, in agreement with the literature.[12] Both I and III and the corresponding *p*-derivatives produce with ninhydrin a yellow colour changing to purple, the fastest colour change being observed for the *o*-compounds.[5]

TABLE 1. R_fS ON PAPER OF BENZYLAMINE DERIVATIVES

Compound	Solvent* 1	2	3	4
(I) *o*-(α-L-Rhamnopyranosyloxy)benzylamine	0·56	0·51	0·59	0·95
p-(α-L-Rhamnopyranosyloxy)benzylamine	0·50	0·41	0·52	0·95
(III) *o*-Hydroxybenzylamine	0·69	0·64	0·68	0·97
p-Hydroxybenzylamine	0·56	0·54	0·56	0·95

* 1 and 2: *n*-BuOH–HOAc–H_2O, (12:3:5) and (4:1:1) (v/v); 3: *iso*ProH–conc. NH_3–H_2O (8:1:1) (v/v); 4: PhOH–H_2O–conc. NH_3 (120:3:1) (v/v); chromatography by descent on Whatman No. 1 paper.

TABLE 2. R_fS ON SILICA GEL PLATES OF BENZYLAMINE DERIVATIVES

Compound	Solvent* 1	2	3	4	5	6
(II) N-[*o*-(*O*-triacetyl-α-L-rhamnopyranosyloxy) benzyl]acetamide	—	—	0·56	0·51	0·69	0·64
N-[*p*-(*O*-triacetyl-α-L-rhamnopyranosyloxy) benzyl]acetamide	—	—	0·47	0·43	0·61	0·56
(I) *o*-(α-L-Rhamnopyranosyloxy)benzylamine	0·51	0·44	—	—	—	—
(III) *o*-Hydroxybenzylamine	0·36	0·34	—	—	—	—

* 1 and 2: Pyridine–water (5:1) and (1:5) (v/v); 3 and 4: Et_2O–EtOH (5:1) and (7:1) (v/v); 5 and 6: $CHCl_3$–EtOH (5:1) and (7:1) (v/v).

Hydrolysis of I with HCl resulted in the production of III and L-rhamnose (IV). III was isolated in the crystalline state from the hydrolysis mixture whereas IV was isolated as the crystalline 2,4-dinitrophenylhydrazone (cf. Ref. 6).

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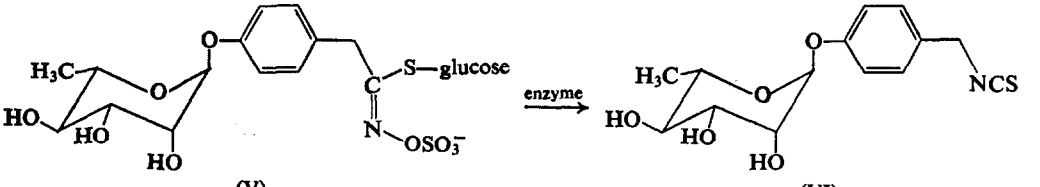
The formulation of the glycosidic bond in I as α-pyranosyloxy (as in all naturally occurring rhamnosides) is in agreement with the NMR spectra of I and II. The negative rotations of I and II also indicate an α-bond.[6] Final corroboration of the configuration has been obtained by a stereospecific synthesis of I and II.

The synthesis of III and its sodium salt was performed as described in the literature.[13] I was synthesized by the reaction of the sodium salt of III with *O*-triacetyl-α-L-rhamnopyranosyl bromide (prepared from L-rhamnose tetraacetate[14]), followed by deacetylation in basic solution. Reaction of this bromide with methanol results mainly in the production of methyl α-L-rhamnopyranoside.[15] This is in agreement with the observation that pyranosyl bromides with an acyloxy group at C_2 *trans* to the bromine atom at C_1, on reaction with OH-groups, produce glycosides with the same configuration as the bromides.[15–17] The small yield of I obtained is due partly to reaction between the bromide and methanol, serving as a solvent and partly to hydrolysis of the glycosidic bond during isolation.

DISCUSSION

The two amines I and III have not been previously encountered in nature and bear no close structural relationship to other natural products. It may however be of interest to compare their occurrence with the occurrence of glucosinolates in *Reseda odorata* L.

A number of amines have been identified in plants containing structurally related glucosinolates. Thus *Sinapis alba* L. (Cruciferae) contains *p*-hydroxybenzylamine and *p*-hydroxybenzylglucosinolate, and *Moringa oleifera* Lam. (syn. *M. pterygosperma* Gaertn.) (Moringaceae) contains benzylamine and benzylglucosinolate.[3] From seeds of *M. oleifera* Lam. has been isolated a glucosinolate with a *p*-(α-L-rhamnopyranosyloxy)benzyl side-chain (V), which on enzymatic hydrolysis produces *p*-(α-L-rhamnopyranosyloxy)benzyl isothiocyanate (VI).[6]



(V) (VI)

It has been observed that *R. odorata* L. contains glucosinolates that can be transformed into thioureas with chromatographic properties similar to, but not identical with, those of the thiourea derivative of V.[18] Further elucidation of the relationship between glucosinolates and amines must await an extension of our present knowledge about the catabolism of glucosinolates in intact plants.[19] The structural resemblance of III and pyridoxamine may be of biological significance, especially because III easily affords Schiff bases.[20]

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EXPERIMENTAL

Isolation of the Amines

Flowers of *Reseda odorata* L. were harvested and stored at −20°. The plant material was homogenized with CCl_4 in a Waring Blendor and defatted twice with CCl_4 (15-l. portions) by refluxing (2 hr), cooling and filtration. After air-drying (residue 2·7 kg) the material was extracted twice with CH_3OH–H_2O (7:3, 15-l. portions) by refluxing (4 hr), cooling and filtration. The combined filtrates were concentrated to a semisolid residue (850 g), which was suspended in water (1·2 l.) and filtered. The filtrate was passed through a column of Amberlite IR 120 (H^+ form; 3·5 × 90 cm), the resin washed with water (25 l.) and elution performed with 2 N NH_4OH (17 l.). The ammonia eluate was concentrated to a semisolid residue (41·8 g), which was dissolved in water (500 ml) and applied to a strongly basic ion-exchange resin in the acetate form (Amberlite IRA 400, 3·2 × 40 cm). Amines, neutral and basic amino acids were not retained but washed out with water (7 l.). The effluent was concentrated to dryness (26·7 g), the residue was dissolved in water (100 ml) and applied to a strongly acid ion-exchange resin in the hydrogen form (Dowex 50WX 8, 200–400 mesh, 2·3 × 35 cm). The column was rinsed with water (10 l.) and neutral amino acids were eluted with pyridine (1 N, 3 l.). Amines and basic amino acids were eluted with 2 N NH_4OH. The eluate was collected in 20-ml fractions. Fractions 6–40 contained III, fractions 30–115 contained I.

Fractions 6–30 were combined and evaporated to dryness (210 mg). III was isolated from the residue by preparative paper chromatography (yield 127 mg) followed by adsorption on a column of Dowex 50WX 8 (200–400 mesh, H^+ form, 0·7 × 10 cm) and elution with 1 N NH_4OH (yield 83 mg). Final purification was achieved by sublimation (60° and 0·02 mm Hg) to yield 55 mg of colourless needles (m.p. 129–129·5°, literature value[7] 126–127°).

Fractions 30–115 were combined and evaporated to dryness (840 mg). The residue was dissolved in water and transferred to a carbon column (Darco G 60, deactivated with stearic acid, 2 × 1 cm). The column was washed with water and eluted with 96% ethanol (180 ml). Further purification of I was achieved by preparative paper chromatography to produce I in the form of an acetic acid salt as a colourless amorphous solid (530 mg). I was liberated by use of Dowex 50 (H^+ form). Evaporation of the ammonia eluate from the resin yielded I as a colourless amorphous solid $[\alpha]_D^{25}$ − 43·8° (*c*. 0·4, H_2O).

Preparation of the Hydrogen Carbonate of Natural I

I (60 mg) was dissolved in water containing CO_2. Concentration of the solution furnished the carbonate as colourless needles (58 mg, 80%), $[\alpha]_D^{25}$ − 39·7° (*c*. 0·2, H_2O). (Found: C, 50·52; H, 6·63; N, 4·05. $C_{13}H_{19}NO_5$, H_2CO_3 required: C, 50·45; H, 6·39; N, 4·23%.)

Attempts to form salts of I with HCO_2H, oxalic acid, HCl, and H_2SO_4 failed.

Preparation of N-[o-(O-triacetyl-α-L-rhamnopyranosyloxy)benzyl]Acetamide from Natural I

On treatment for 24 hr with Ac_2O (pyridine at room temp.), I furnished the acetate as a colourless semi-crystalline residue (118 mg, 96%). (Found: C, 56·00; H, 6·26; N, 3·12; H_2O, 3·2 (the water was determined as weight loss when the sample was dried over P_2O_5 at room temp.; the water was taken up again, when the sample was exposed to the atmosphere). $C_{21}H_{27}NO_9$, 4/5H_2O required: C, 55·82; H, 6·38; N, 3·10; H_2O, 3·19%.) $[\alpha]_D^{25}$ − 26·4° (*c*. 6·6, $CHCl_3$).

Hydrolysis of Natural I

A solution of I (250 mg) in HCl (10 ml, 6 N), was heated for 48 hr at 90°, cooled and evaporated to dryness. The residue was dissolved in H_2O and applied to a column of Dowex 50WX 8 (200–400 mesh, H^+ form, 0·7 × 10 cm) which was washed with water (15 ml) and eluted with pyridine (1 N, 5 ml) and then 1 N NH_4OH (15 ml).

The water effluent was evaporated to dryness and the rhamnose present was purified by ion exchange and preparative paper chromatography. The colourless amorphous solid obtained (7 mg) (identical in R_f to rhamnose) yielded the crystalline 2,4-dinitrophenylhydrazone of L-rhamnose (4·7 mg). $[\alpha]_D^{25}$ − 54·0° (*c*. 0·2, ethyl acetate), m.p. 167° (literature value[6] 166°). An authentic sample, produced from L-rhamnose in the same way, had $[\alpha]_D^{23}$ − 53·4° (*c*. 0·2, ethyl acetate), m.p. 166–167°. The i.r. spectra of the two samples were identical.

The ammonia eluate from the hydrolysis mixture was evaporated to dryness (33 mg). III was purified by preparative paper chromatography, passage through a strongly acid ion-exchange resin, and sublimation as described under the isolation of III. III was obtained as colourless crystals (13 mg), m.p. 128–129°.

Synthesis of III

The amine III and its sodium salt were synthesized from salicylamide as described in the literature.[13] After purification by sublimation, a 55% yield of III was obtained, m.p. 129–129·5°. (Found: C, 68·10; H, 7·46; N, 11·23. Calc. for C_7H_9NO: C, 68·27; H, 7·38; N, 11·38%).

Synthesis of O-Triacetyl-(α-L-rhamnopyranosyl)bromide

L-Rhamnose tetraacetate was prepared[6] from L-rhamnose and acetic anhydride in pyridine, yield 98%. The bromide was prepared from the tetraacetate,[14] yield 66%, m.p. 68–69° (literature value[15] 64–67°), $[\alpha]_D^{25}$ − 181° ($CHCl_3$). (Found: C, 41·24; H, 4·90; Br, 21·82. Calc. for $C_{12}H_{17}O_7Br$: C, 40·81; H, 4·85; Br, 22·63%.)

Synthesis of I

A solution of the sodium salt of III (8·70 g) in methanol (10 ml) was added to a solution of *O*-triacetyl-(α-L-rhamnopyranosyl)bromide (25·5 g) in acetonitrile (300 ml) in the course of 3 min. The mixture was stirred for 15 min at room temp., 2 N NH_4OH (10 ml) added, the solution was evaporated to dryness, and the residue was extracted with water (7 × 50 ml). Evaporation of the combined extracts yielded a semisolid material (10·8 g) containing a mixture of I and III in the proportion 2:1 as demonstrated by paper chromatography. Purification of I was achieved by use of ion-exchange resins and preparative paper chromatography. I was obtained as a colourless amorphous solid (560 mg, 3·5%), $[\alpha]_D^{25}$ − 46·3° (*c.* 0·2, H_2O).

Preparation of the Hydrogen Carbonate of Synthetic I

The hydrogen carbonate was prepared from 60 mg of synthetic I as described for the natural material, yield 53 mg (70%), $[\alpha]_D^{25}$ − 36·8° (*c.* 0·2, H_2O). (Found: C, 49·94; H, 6·33; N, 4·22%.)

Preparation of II from synthetic I

The synthesis was performed on 202 mg of I as described above for the synthesis of naturally derived material, yield 310 mg (94%), $[\alpha]_D^{25}$ − 28° (*c.* 1·2, $CHCl_3$). (Found: C, 55·87; H, 6·29; N, 3·10; H_2O, 3·3%.)

Demonstration of the Presence of I and III in the Native Plant Material Using Mild Methods for Extraction

Flowers of *R. odorata* L. (3 g) were homogenized with a mixture of CCl_4 and water at room temp. After filtration and partial concentration of the water phase at room temp., paper chromatography showed the presence of both I and III.

Paper Chromatography and Thin-layer Chromatography

The R_fs obtained by paper chromatography are presented in Table 1, those obtained by thin-layer chromatography in Table 2. Preparative paper chromatography was performed on Whatman paper No. 3 MM by the descending technique with solvent 1 in Table 1.

General Methods and Instrumentation

The identity of natural and synthetic I, II, and III was established by R_f, u.v., i.r. NMR and mass spectral comparisons. M.p.s were determined on an Anschütz–Herzberg apparatus with fully immersed thermometer. I.r. spectra were measured in KBr discs. Microanalyses were performed by Mr. G. Cornali.

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