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SYNTHESIS AND DIGESTIBILITY INHIBITION OF DIARYLHEPTANOIDS: STRUCTURE–ACTIVITY RELATIONSHIP

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Abstract—(±)-5-Hydroxy-1,7-bis-(4′-hydroxyphenyl)-3-heptanone (**2a**), (±)-5-hydroxyl-1-(4′-hydroxyphenyl)-7-phenyl-3-heptanone (**2b**), (±)-5-hydroxy-7-(4′-hydroxyphenyl)-1-phenyl-3-heptanone (**2c**), and (±)-5-hydroxy-1,7-bis-(phenyl)-3-heptanone (**2d**) have been synthesized to study the structure–activity relationship regarding digestibility inhibition in vitro in cow rumen fluid. The activities were compared with the activity of chiral (*S*)-**2a** and its glucoside platyphylloside (**1**), isolated from *Betula pendula*. Compound **2a** was slightly less active, **2b** and **2c** were more active, and **2d** was less active than (*S*)-**2a** and platyphylloside.

Key Words–Platyphylloside, 5-hydroxy-3-platyphyllone, centrolobol, phenols, rumen fluid, birch bark, *Betula pendula*.

INTRODUCTION

Birch (*Betula pendula* Roth.) bark contains a phenolic glucoside, platyphylloside (**1**), that has been shown to reduce the in vitro digestibility of hay in goat and sheep rumen fluid up to 50% (Sunnerheim-Sjöberg et al., 1988).

Platyphylloside was first isolated by Terasawa et al. (1984) from *Betula platyphylla*. Diarylheptanoid glycosides have also been found in other species such as *Acer* spp. (Nagai et al., 1990; Nagumo et al., 1996), *Alnus* spp. (Sasaya and Izumiyama, 1974; Sasaya, 1985; Nomura et al., 1981; Ohta et al., 1984), *Curcuma xanthorrhiza* (Claeson et al., 1996), *Alpinia blephaocalyx* (Kadota et al., 1996), and *Zingiber officinale* (Kikuzaki and Nakatani, 1996). Many of

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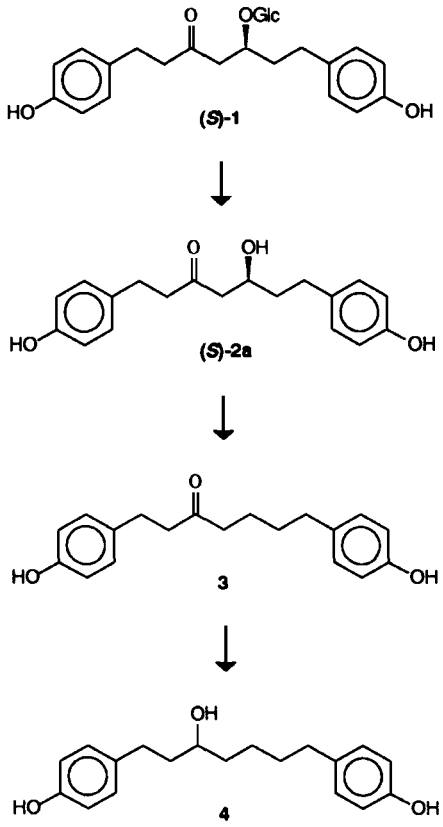


SCHEME 1. Metabolism of platyphylloside [(*S*)-**1**] in vitro in sheep rumen fluid. Platyphylloside [(*S*)-**1**] → (5*S*)-hydroxy-3-platyphyllone [(*S*)-**2a**] → 3-platyphyllone (**3**) → centrolobol (**4**).

them possess biological activities, such as antiinflammatory and antihepatotoxic effects, and some inhibit prostaglandin biosynthesis (Claeson et al., 1996, and references therein).

The metabolism of platyphylloside in vitro has been studied by Sunnerheim-Sjöberg and Knutsson (1995). When platyphylloside was added to hay and incubated together with rumen fluid, the metabolism was deduced to occur in three steps. First, hydrolysis of the glycosidic bond results in the hydroxy ketone 5-hydroxy-3-platyphyllone [(*S*)-**2a**]. Then, a reduction of the hydroxy function to a methylene group gives the ketone 3-platyphyllone (**3**). The carbonyl group is further reduced to a hydroxy functon leading to the alcohol centrolobol (**4**) (Scheme 1).

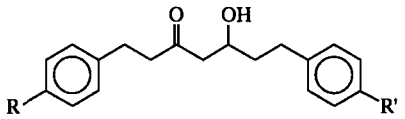


FIG. 1. Synthesized diarylheptanoids. **2a:** R = OH, R′ = OH, 5-hydroxy-1,7-bis-(4′-hydroxyphenyl)-3-heptanone; **2b:** R = OH, R′ = H, 5-hydroxy-1-(4′-hydroxyphenyl)-7-phenyl-3-heptanone; **2c:** R = H, R′ = OH, 5-hydroxy-7-(4′-hydroxyphenyl)-1-phenyl-3-heptanone; **2d:** R = H, R′ = H, 5-hydroxy-1,7-bisphenyl-3-heptanone.

The aim of this work was to synthesize four racemic diarylheptanoids (Figure 1) differing in the substituents on the aromatic rings, to study the structure–activity relationship regarding digestibility inhibition in vitro and to compare the activities to the activities of the chiral (*S*)-**2a** and its glucoside platyphylloside.

Linear diarylheptanoids containing the β-ketol function have been synthesized previously via direct C-alkylation by using diethyl phosphorocyanidate followed by Grignard reactions with aldehydes (Kato et al., 1984). In this paper, several analogues, **2a–d**, were synthesized via an aldol reaction. Our method provides a shorter, versatile route to this class of compounds.

METHODS AND MATERIALS

Chromatography

HPLC was performed on a Waters chromatograph equipped with a photo diode array detector. The column was a LichroCart 125-4 Lichrospher 100 RP-18 (5 μm) (Merck). The mobile phase consisted of solvent A: 0.01 M Ammonium formiate buffer, pH 3.4; and solvent B: acetonitrile. The gradient program was 0–10 min linear gradient from 50% to 70% B. The flow rate was 1 ml/min. TLC was performed on Merck HF-254 silica gel plates. For flash column chromatography, Merck Kieselgel 60 (230–400 mesh) was used.

Spectroscopy

^{1}H NMR (400 MHz) and ^{13}C NMR (100.5 MHz) NMR spectra were recorded on a Varian Unity 400 by using the solvent signals ($CDCl_3$ or CD_3OD) as internal standards.

Digestibility Experiment

In vitro organic matter digestibility (IVOMD) was determined according to standard methods originally described by Lindgren (1979). Rumen fluid was

sampled from a cannulated cow fed on 40% hay and 60% concentrate. The samples (see below) were incubated for 96 hr in 50 ml of a mixture of buffer (pH 6.8 ± 0.1) and rumen fluid (50 : 1) maintained under anaerobic conditions at 38°C in a water bath (Lindgren, 1979). After incubation, filtration, and washing, the amount of insoluble organic matter was determined by the difference in weight between dried (103°C over night) and deashed (500°C for 1.5 hr) solid residue. Reduction of digestibility was calculated by using equation 1:

$$(IVOMD_{ctrl} - IVOMD_{sample})/IVOMD_{ctrl} \quad (1)$$

Sample Preparation. To each tube, 500 mg milled hay and 0.050 mmol of the compound to be tested dissolved in 96% aqueous ethanol was added and mixed. (The concentration of the tested compounds in hay was, thus, about five times higher compared to the concentration of platyphylloside found in birch twigs and about the same as estimated in birch bark.) The control samples were treated the same way but with hay and solvent only. Three replicates of each sample were made. The solvent was allowed to evaporate at 40°C for 16 hr (all of the tested compounds were stable at this temperature). To each tube, 50 ml of buffer with 2% rumen fluid was added. After incubation (96 hr at 38°C), the hay residues were separated from the liquid by filtration. Ethanol (60 ml) was added to the different samples to stop enzymatic reactions during storage. Before HPLC analysis, the solvent was evaporated, and the residue was dissolved in water. The aqueous phase was extracted with ether/pentane (50 : 50) and washed with water and brine. The organic phase was dried with $MgSO_4$, and the solvent was evaporated.

Source of Chemicals

Platyphylloside [(5*S*)-hydroxy-1,7-bis-(4′-hydroxyphenyl)-3-heptanone-5-*O*-β-D-glucopyranoside (**1**)] was isolated from the inner bark of *Betula pendula* according to Smite et al. (1993). (5*S*)-Hydroxy-3-platyphyllone [(5*S*)-hydroxy-1,7-bis-(4′-hydroxyphenyl)-3-heptanone, (*S*)-**a**] was obtained by enzymatic hydrolysis of platyphylloside (Sunnerheim-Sjöberg et al., 1988).

4-(4′-Pyranyloxyphenyl)-2-butanone (***5a***). To a stirred solution of 4-(4′-hydroxyphenyl)-2-butanone (7.50 g, 46 mmol) and freshly distilled dihydropyran (10.5 ml, 113 mmol) in dry CH_2Cl_2 (40 ml) was added *p*-toluenesulfonic acid (monohydrate) (0.085 g, 0.44 mmol) in one portion. The mixture was stirred at room temperature for 1 hr. $NaHCO_3$ (aq) was added, and the mixture was stirred for 30 min. The mixture was extracted with Et_2O, dried over $MgSO_4$, and the solvent was evaporated to give a brown oil that was further purified by flash column chromatography (pentane–ether, 70 : 30) to give a colorless liquid (10.396 g, 92%). ^1H NMR was in accordance with literature (Schuster and Polowczyk, 1966).

Methyl 3-(4′-pyranyloxyphenyl)propanoate (***9***). To a stirred solution of

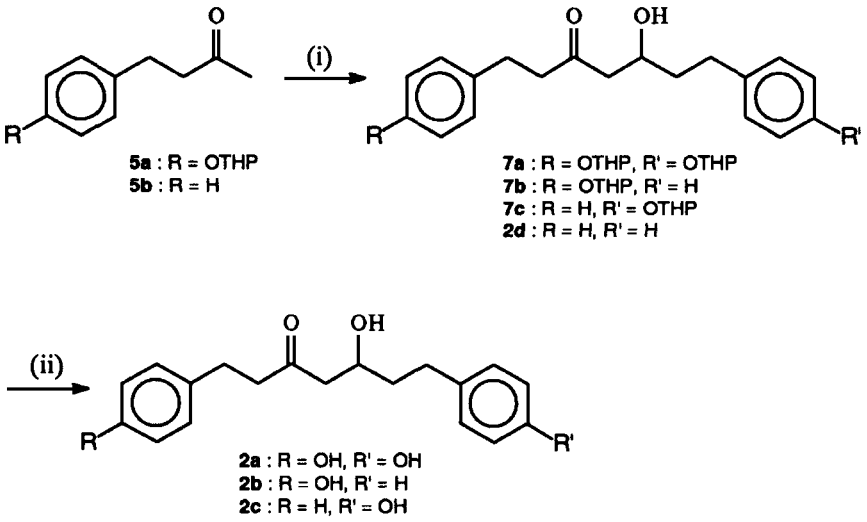
methyl 3-(4′-hydroxyphenyl)propanoate (**8**) (2.02 g, 11.2 mmol) was freshly distilled dihydropyran (2.6 ml, 28 mmol) in dry CH_2Cl_2 (10 ml) was added *p*-toluenesulfonic acid (monohydrate) (0.021 g, 0.11 mmol) in one portion. The mixture was stirred at room temperature for 1.5 hr. $NaHCO_3$ (aq) was added, and the mixture was stirred for 30 min. The mixture was extracted with Et_2O, dried over $MgSO_4$, and the solvent was evaporated to give a brown oil, which was further purified by flash column chromatography (pentane–ether, 60 : 40) to give a colorless liquid (3.198 g, 93%). ^{1}H NMR ($CDCl_3$) δ 7.10 (m, 2H), 6.98 (m, 2H), 5.37 (t, J = 3.2 Hz, 1H), 3.91 (m, 1H), 3.66 (s, 3H), 3.60 (m, 1H), 2.89 (m, 2H), 2.60 (m, 2H), 1.95–2.05 (m, 1H), 1.82–1.88 (m, 2H), 1.53–1.71 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 173.4, 155.5, 133.5, 129.1, 116.5, 96.5, 62.0, 51.5, 36.0, 30.4, 25.2, 18.9.

3-(4′-Pyranyloxyphenyl)propanol (***10***). A solution of methyl-3-(4′-pyranyloxyphenyl)propanoate (**9**) (11.445 g, 43.4 mmol) in dry diethyl ether (20 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (2.472 g, 65.0 mmol) in diethyl ether (100 ml) at room temperature. The mixture was left to stir under nitrogen atmosphere at room temperature for 1.5 hr. $Na_2SO_4 \times 10\ H_2O$ was added carefully in portions until no gas evolved. The mixture was filtered, the solid washed with ether, the filtrate dried with $MgSO_4$, and the solvent evaporated to give a colorless liquid (1.522 g, 97%). The product was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 7.10 (m, 2H), 6.98 (m, 2H), 5.37 (t, J = 3.4 Hz, 1H), 3.93 (m, 1H), 3.65 (t, J = 6.4 Hz, 2H), 3.60 (m, 1H), 2.64 (m, 2H), 1.95–2.07 (m, 1H), 1.82–1.92 (m, 2H), 1.57–1.73 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 155.3, 134.8, 129.2, 116.5, 96.6, 62.2, 62.0, 34.3, 31.2, 30.4, 25.2, 18.8.

3-(4′-Pyranyloxyphenyl)propanal (***6a***). Pyridinium chlorochromate (0.660 g, 3.1 mmol) and sodium acetate (0.051 g, 0.6 mmol) were suspended in anhydrous CH_2Cl_2 (5 ml), and 3-(4′-pyranyloxyphenyl)propanol (**10**) (0.530 g, 2.2 mmol) in CH_2Cl_2 (5 ml) was added in one portion to the stirred solution. After 2 hr, dry ether was added, and the supernatant was decanted from the black insoluble residue. The residue was washed with ether, the combined organic phases were filtered through silica, and the solvent was evaporated to give a colorless liquid (0.343 g, 67%), which was pure according to ^{1}H NMR. ^{1}H NMR ($CDCl_3$) δ 9,81 (m, 1H), 7.07–7.13 (m, 2H), 6.98 (m, 2H), 5.38 (t, J = 3.2 Hz, 1H), 3.91 (m, 1H), 3.59 (m, 1H), 2.90 (m, 2H), 2.75 (m, 2H), 1.95–2.05 (m, 1H), 1.82–1.88 (m, 2H), 1.54–1.73 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 201.7, 155.5, 133.3, 129.1, 116.6, 96.5, 62.0, 45.5, 30.4, 27.4, 25.2, 18.8.

General Procedure for Aldol Condensation: Synthesis of Compound 7a (Scheme 2)

To a flame-dried flask under nitrogen atmosphere, diisopropylamine (0.21 ml, 1.5 mmol) was added followed by dry THF (3.0 ml). After the mixture was cooled to −78°C, *n*-butyllithium (1.6 M, 0.89 ml, 1.4 mmol) was added, and



SCHEME 2. (i) 1) LDA (lithium diisopropylamide), 2) **6a** or **6b** (Figure 2), 3) H^+; (ii) HOAc–THF–H_2O (2:2:1).

the mixture was stirred for 35 min. A solution of 4-(4′-pyranyloxyphenyl)-2-butanone (0.321 g, 1.3 mmol) in THF (3.0 ml) was added slowly. After stirring for 30 min, a solution of 3-(4′-pyranyloxyphenyl)propanal (0.304 g, 1.3 mmol) in THF (2.0 ml) was added dropwise, and the mixture stirred for another 2 hr. The reaction was quenched at −78°C by careful addition of HCl (1 M, 5.0 ml). The reaction mixture was allowed to warm to room temperature. The mixture was extracted with ether, washed with brine, and dried with $MgSO_4$. The solvent was evaporated to give the crude product, which was purified by flash column chromatography (ether–pentane) to give a colorless oil.

Deprotection of THP

The deprotection of THP was done according to Bernady et al. (1979). Spectral data were consistent with those previously reported [**a** (Sunnerheim-Sjöberg and Knutsson, 1995), **2c** (Kiuchi et al., 1992), **2d** (Asakawa, 1970), **2b** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16–7.32 (m, 5H), 7.02 (m, 2H), 6.74 (m, 2H), 4.05 (oct, J = 4.0 Hz, 1H), 3.16 (d, J = 4.0 Hz, OH), 2.75–2.86 (m, 3H), 2.62–2.73 (m, 3H), 2.54 (m, 2H), 1.76–1.87 (m, 1H), 1.62–1.72 (m, 2H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 211.7, 154.3, 141.5, 132.0, 129.2, 128.3, 125.8, 115.4, 67.2, 49.0, 45.2, 37.8, 31.6, 28.5. (Kiuchi et al., 1992)].

RESULTS AND DISCUSSION

Synthetic Route

The aldol condensation with the ketones (**5a,b**) and aldehydes (**6a,b**) produced the diarylheptanoides (**7a–c** and **2d**) containing the (±)-β-ketol skeleton as shown in Scheme 2. The phenolic moieties were protected as tetrahydropyranyl (THP) ethers. The yields from the aldol condensation reactions can be seen in Table 1.

The aldehyde **6a** (Figure 2) was prepared according to Scheme 3. Protection of the commercially available ester **8** with dihydropyrane (DHP) followed by reduction using lithium aluminum hydride ($LiAlH_4$) and oxidation using pyridinium chlorochromate (PCC) (Corey and Suggs, 1975) produced the aldehyde **6a**.

Digestibility Studies: Structure–Activity Relationship

Platyphylloside [(*S*)-1], (*S*)-**2a**, racemic **2a**, and the three analogs, **2b–2d** (Figure 1), were tested for digestibility inhibition in vitro in cow rumen fluid. The results are presented in Figure 3.

Glycoside–Aglycone. It is known from earlier studies (Sunnerheim-Sjöberg and Knutsson, 1995) that the glycosidic bond in platyphylloside is completely hydrolyzed in vitro within 6 hr in rumen fluid to (*S*)-**2a**. Moreover, the digestibility-reducing activity is correlated with the concentration of the metabolite (**4**) formed by reduction of (*S*)-**2a** (Sunnerheim-Sjöberg et al., 1988). Thus, the aglycone and its glucoside, platyphylloside, were expected to behave identi-

TABLE 1. ALDOL CONDENSATION OF KETONES **5a,b** AND ALDEHYDES **6a,b**

Entry	Ketone	Aldehyde	Product	Yield (%)
1	**5a**	**6a**	**7a**	71
2	**5a**	**6b**	**7b**	67
3	**5b**	**6a**	**7c**	72
4	**5b**	**6b**	**2d**	63

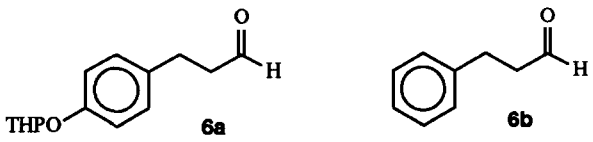
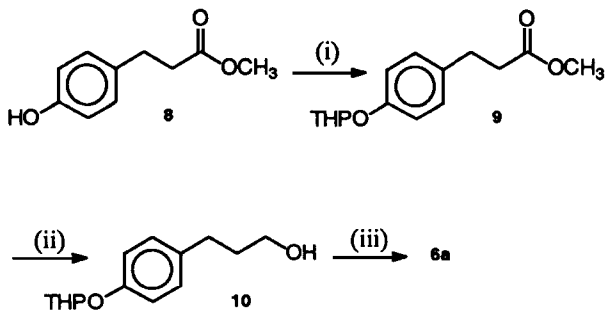


FIG. 2. **6a** = 3-(4′-Pyranyloxyphenyl)propanal, **6b** = 3-phenylpropanal.



SCHEME 3. (i) DHP, *p*-toluenesulfonic acid, CH_2Cl_2, 93%; (ii) $LiAlH_4$, Et_2O, 97% (iii) PCC, $NaCO_2CH_3$, CH_2Cl_2, 67%.

cally after a short incubation time. The results confirmed these expectations since the platyphylloside was almost as active as (*S*)-**2a** (32% and 35%, respectively, Figure 3). Furthermore, HPLC analysis of the rumen fluid after incubation showed, as expected from previous results (Sunnerheim-Sjöberg and Knutsson, 1995), that only two metabolites, **3** and **4**, were formed from both platyphylloside and (*S*)-**2a**. The concentration ratios of **4** and **3**, were 36 : 64 and 38 : 62 from **1** and (*S*)-**2a**, respectively.

Stereochemistry–Activity. The racemic mixture of **2a** was slightly less active than the isolated, optically active one, (*S*)-**2a**, 28% and 35%, respectively (Figure 3). The chromatograms (Figure 4a and 4b) show that (*S*)-**2a** is totally metabolized, while the racemic **2a** is not.

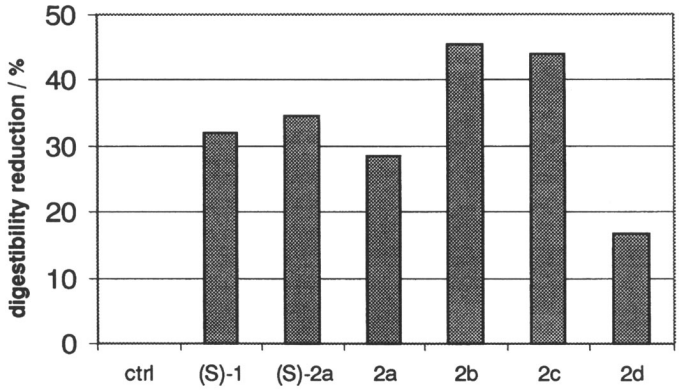


FIG. 3. Digestibility reduction after incubation for 96-hr. ctrl = control, (*S*)-**1** = platyphylloside, (*S*)-**2a** = (5*S*)-hydroxy-1,7-bis-(4′-hydroxyphenyl)-3-heptanone, **2a–d**, see Figure 1. Standard deviations: 0.75, 3.26, 1.07, 1.03, 0.46, 0.90, and 0.95, respectively.

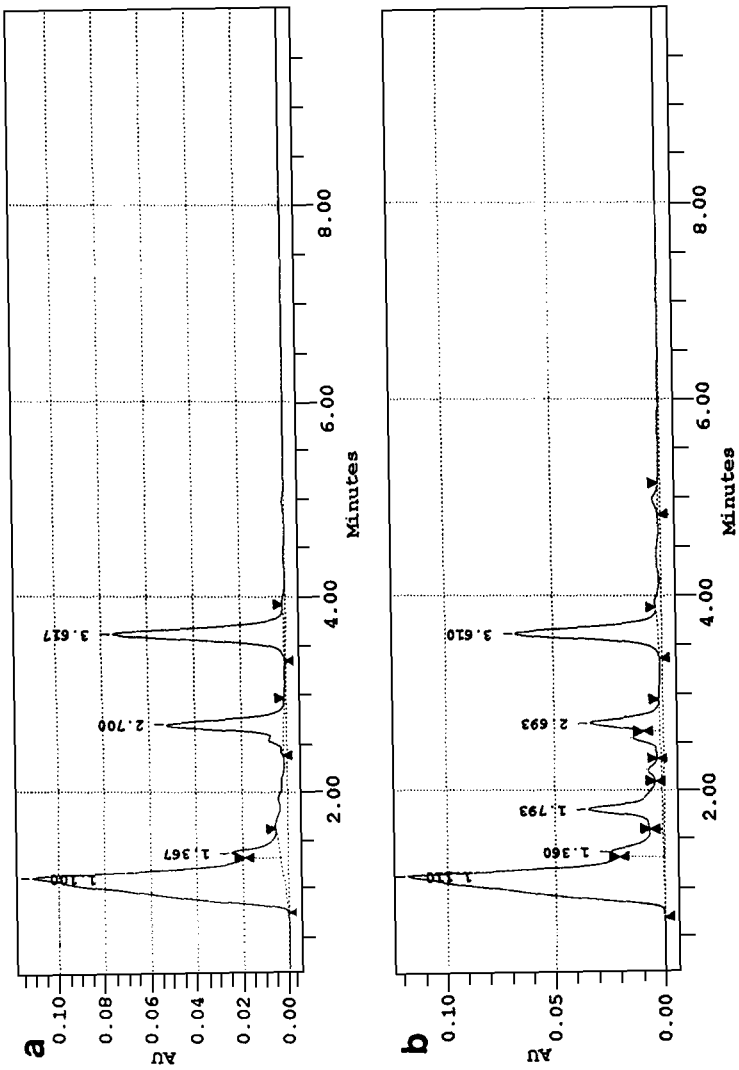
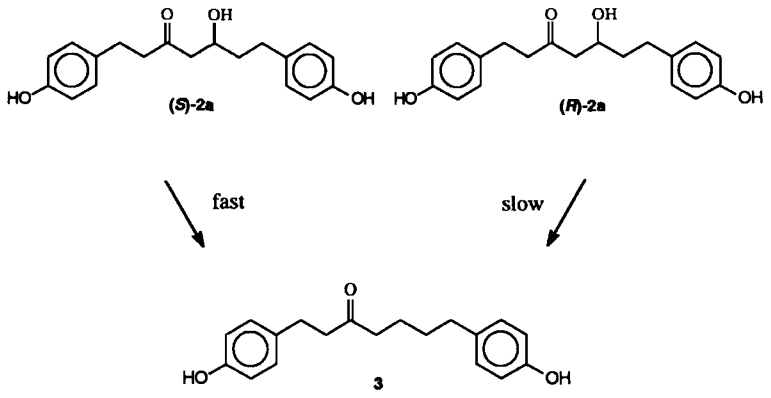


FIG. 4. (a) HPLC of rumen fluid after incubation with (5*S*)-hydroxy-3-platyphyllone [(*S*)-**2a**], R_f = 2.7 min centrolobol (**4**), R_f = 3.6 min 3-platphyllone (**3**). (b) HPLC of rumen fluid after incubation with (±)-5-hydroxy-3-platyphyllone (**2a**), R_f = 1.8 min 5-hydroxy-3-platphyllone, R_f = 2.7 min centrolobol (**4**) R_f = 3.6 min 3-platyphyllone (**3**).



SCHEME 4. In vitro metabolism of (*S*)-**2a** and (*R*)-**2a** in rumen fluid.

Estimation from peak areas shows that only about three fourths of the racemic compound has been metabolized. We assume that this is due to slower reduction of (*R*)-**2a** than to the *S*-enantiomer in the rumen fluid (Scheme 4).

Active Metabolite. The estimated relative areas of the peaks from centrolobol (**4**) in the chromatograms in Figure 4a and 4b are correlated to the activity. This supports the results from a previous study (Sunnerheim-Sjöberg and Knutsson, 1995) indicating centolobol (**4**) as the active metabolite.

Aromatic Substitution–Activity. The two derivatives lacking one phenolic hydroxyl group (**2b** and **2c**) possess a higher activity than the naturally occurring platyphylloside and its aglycone, while the nonphenolic derivative (**2d**) is less active (Figure 3).

SUMMARY

IVOMD of two isolated and four synthetic diarylheptanoids was studied for structure–activity relationship. Both the hydroxylation pattern in the aromatic rings and the stereochemistry were of importance for the activity.

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