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ENZYMATIC HYDROLYSIS OF PLUMIERIDE TO PLUMIERIDINE

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Key Word Index—Iridoids; iridoid glucoside; hydrolysis; plumieride; plumieridine; plant growth inhibitor.

Abstract—The hydrolysis of plumieride to form the aglycone plumieridine was catalysed by a commercial preparation of cellulase from *Aspergillus niger*.

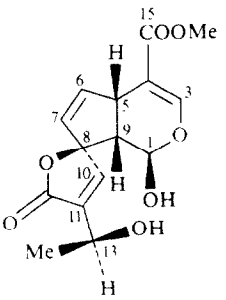
INTRODUCTION

Since the pioneering structural investigation of plumieride done by Schmid *et al.* [1, 2] more than 250 iridoids have been found in different plant families [3]. Although this class of cyclopentan-(c)-pyran monoterpenoids is mainly of pharmaceutical interest, the plant growth inhibiting activity of the principal iridoid glycoside plumieride has been reported [4]. To date, all efforts to obtain the corresponding aglycone of plumieride by acidic [1, 2] as well as enzymatic hydrolysis using helicase [1], emulsin or hemicellulase [5] have failed. In this paper, we report the smooth enzymatic preparation of the aglycone (**1**), for which we propose the name plumieridine.

RESULTS AND DISCUSSION

For the investigation of plumieride hydrolysis a cellulase preparation from *Aspergillus niger* was used. Preliminary experiments demonstrated (TLC) that at pH 5 and 20°, plumieride was quantitatively converted to four compounds with R_f 0.61, 0.65, 0.70 and 0.77 (plumieride R_f 0.46) in the presence of the cellulase. This finding indicated that the unstable aglycone underwent further reactions under the weak acidic conditions of the assay. To determine whether cellulase was able to hydrolyse plumieride under neutral conditions, the pH-dependence of plumieride hydrolysis with cellulase was determined by using the glucose oxidase-peroxidase reaction [6]. Although maximum hydrolytic activity was found at pH 3, even at pH 7 30% of the applied plumieride was hydrolysed to form plumieridine (**1**). As a result of this finding, the hydrolysis was carried out at pH 7 in a two-phase system with ethyl acetate as the upper layer (TLC-monitoring). By means of this *in situ* extraction, further reactions were avoided and only the formation of **1** was observed.

Plumieridine (**1**), $C_{15}H_{16}O_7$ (high resolution mass spectrometry) was obtained as crystals from Et_2O–EtOAc–*n*-hexane, mp 161–163° (dec.). Its mass spectrum (10–16 eV) contained highly diagnostic peaks at *m/z* (rel. int.) 308 $[M]^+$ (8), 290 $[M-H_2O]^+$ (43), 272 $[M-2H_2O]^+$ (20), 246 $[M-CO_2]^+$ (97), 228 $[M-CO_2-H_2O]^+$ (73), 203 [228–15]$^+$ (100), 139 (72) and 115 (99). This fragmentation pattern was nearly the same as that found earlier for the parent glucoside, plumieride [7]. The anion mass spectrum (10–16 eV) had, in addition to a peak at *m/z* 336 [M + CO] (11), prominent ions at *m/z* 308 $[M]^-$ (44), 290 $[M-H_2O]^-$ (98), 272 $[M-2H_2O]^-$ (23) and 246 $[M-CO_2]^-$ (100). In addition, the ^1H NMR spectrum was in full agreement with the expected structure **1**.



1

In its plant growth inhibiting properties, plumieridine (**1**) showed the same effect of counteracting the gibberellin-induced growth, in the dwarf rice bioassay as shown earlier [4] for its glucoside, plumieride. Thus the structural feature responsible for the observed inhibiting activity seems to be the unsaturated lactone group, as is the case for other plant constituents with such a function [8].

EXPERIMENTAL

Mps are corr. TLC: Si gel, $CHCl_3$–MeOH (8:2), detection with vanillin–H_3PO_4 10 min at 110°.

Determination of the pH *dependence of the plumieride hydrolysis.* To 100 μl buffer (citrate–HCl, pH 1.3–3.0, and citrate–phosphate, pH 3.0–7.8) and 20 μl (67 μg) cellulase (pract., Ferak) was added 50 nmol plumieride in 20 μl H_2O and the mixture incubated at 37°. After addition of 1 ml glucose oxidase-peroxidase reagent the degree of hydrolysis was determined [6].

Plumieridine (**1**) *from plumieride.* To 4 ml of a soln of 13.4 mg cellulase in 0.1 M McIlvain buffer (pH 7), 47 mg plumieride and 4 ml EtOAc were added and the soln shaken for 16 hr at 20°. The aq. layer was extracted twice with 2 ml EtOAc. Evaporation of the combined soln under red. pres. yielded plumieridine (**1**): crystals (Et_2O–EtOAc–*n*-hexane),

mp 161–163°; $[\alpha]_D^{29} - 57.4°$ (EtOAc; $c = 0.406$); R_f 0.70; IR ν_{max}^{nujol} cm^{-1}: 3490, 3300, 3096, 3082, 1745, 1703, 1625, 1152, 1098, 1040, 895, 805, 760; UV λ_{max}^{EtOH} nm (log ϵ): 245 (2.36, sh), 215 (4.19); ^1H NMR (100 MHz, pyridine-d_5 TMS int. standard) δ 7.47 (*d*, $J = 2$ Hz, H-3), 7.29 (*d*, $J = 1$ Hz, H-10), 6.38 (*td*, $J = J' = 6$ Hz, $J'' = 2$ Hz, H-6), 5.33 (*d*, $J = 6$ Hz, H-7), 5.33 (*d*, $J = 4$ Hz, H-1), 4.82 (*br q*, $J = 7$ Hz, $J' = 1$ Hz, H-13), 3.94 (*br q*, $J = 8$ Hz, H-5), 3.53 (*s*, $-COOCH_3$), 3.10 (*q*, $J = 8$ Hz, $J' = 4$ Hz, H-9), 1.47 (*d*, $J = 7$ Hz, CH_3-14). Other spectral data are in the text.

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GERMACRANOLIDES FROM *PIPTOLEPIS LEPTOSPERMOIDES**

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Key Word Index—*Piptolepis leptospermoides*; Compositae; Vernonieae; sesquiterpene lactones; furanoheliangolide; germacranolide.

Abstract—*Piptolepis leptospermoides* afforded, in addition to known compounds, a new eremanthanolide and a germacranolide closely related to piptolepolide.

The small Brazilian genus *Piptolepis* is placed in the subtribe Lychnophorinae [1]. So far the chemistry has supported this assignment, as, in addition to triterpenes derived from lupane, a furanoheliangolide has been isolated [2]. We have now studied the constituents of *Piptolepis leptospermoides* (DC) Sch. Bip. The roots afforded polyisoprene, lupeyl acetate, lupenone, taraxasteryl acetate, taraxasterone and eremanthin (**1**) [3], while the aerial parts gave germacrene D, α-humulene, bisabolene, squalene, lupenone and lupeyl acetate. The polar fractions contained a mixture of small amounts of sesquiterpene lactones which were separated with difficulty into the furanoheliangolides **2** [4] and **3** [5], the eremanthanolides **4** [6], **5** [7] and **6** and the germacranolide **7**. The structures were elucidated by their ^1H NMR spectra.

The spectrum of **6** (Table 1) showed the typical signal of a furanoheliangolide (5.72 *s*), and those of an eremantholide. Most signals were similar to those of eremantholide C [6, 7]. However, the olefinic methyl group was missing. A broadened two-proton singlet at δ 4.39 indicated a 15-hydroxy group. Accordingly, the H-5 signal was shifted downfield (6.31 *dt*), as in the spectrum of a closely-related eremanthanolide from a *Lychnophora* species [7]. The ^1H NMR spectrum of **7** (Table 1) showed the presence of a methylene lactone by the typical downfield doublets at δ 6.32 and 5.70. These protons were coupled with a four-fold doublet at δ 2.94, as was shown by spin decoupling. The latter was further coupled with a three-fold doublet at δ 5.00 and 4.45. Irradiation at δ 5.00 collapsed the double doublet at 2.61 to a doublet and sharpened a broadened doublet at δ 2.15. As these two signals also showed a geminal coupling of 14 Hz partial structure **A** was assigned.

From further decouplings the presence of sequence **B** was deduced. The chemical shifts of H-2 indicated the neighbouring keto group, the position of which

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