Phytochemistry, Vol. 24, No 8, pp. 1841–1842, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

IRIDOIDS FROM *DESFONTAINIA SPINOSA*

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(*Revised received* 10 *December* 1984)

Key Word Index—*Desfontainia spinosa*; Loganiaceae; hallucinogens; iridoids; loganin; loganic acid; loganetin.

Abstract—The leaves of *Desfontainia spinosa* were found to contain the known iridoid glucosides loganin and loganic acid. Loganetin, the aglucone of loganin was also isolated. This compound has not previously been reported as occurring naturally.

INTRODUCTION

Desfontainia spinosa Ruiz and Pavon is a shrub with spiny leaves resembling those of *Ilex europea*. There is some controversy over its taxonomic position and botanists have placed it in the Potaliaceae, Loganiaceae or Desfontainiaceae, the latter two being preferred [1]. The plant is indigenous to the Andes in South America and a tea made from its leaves is said to be used by several Indian groups as a narcotic and hallucinogen [2]. No previous phytochemical work on this plant has been reported apart from alkaloid screening of herbarium material which gave a negative result [2].

RESULTS AND DISCUSSION

Loganic acid was identified by spectral and chromatographic comparison with the literature [4, 5] and with loganic acid obtained by the alkaline hydrolysis of loganin. Its identity was confirmed by its conversion to loganin by methylation.

Loganetin was identified on the basis of comparing its spectral and chromatographic characteristics with the values given in the literature [6, 7] and with loganetin produced by the enzymatic hydrolysis of loganin. The presence of loganin and related iridoids accounts for the bitter taste of infusions made from *Desfontainia* leaves. Their presence has also chemotaxonomic significance in that it justifies the inclusion of the genus in the Loganiaceae.

Loganetin has been prepared synthetically [7] but it has not been reported previously as a naturally occurring compound. It is unusual for iridoids to occur as aglycones, particularly in conjunction with the corresponding glycoside. No evidence for the presence of the aglucone of loganic acid could be found when extracts were examined by TLC and compared with a sample produced by enzymatic hydrolysis. It could be argued that loganetin is an artefact arising from hydrolysis of loganin during the infusion process but this is discounted by the detection of loganetin in cold chloroform extracts of fresh leaves. The presence of these iridoids does not provide an explanation for the alleged CNS activity of this plant since there are no reports of such compounds having this property. The CNS activity of many plants used as hallucinogens or narcotics is due to the presence of alkaloids, especially the indole bases, but these do not seem to be present as the simple screening tests used gave negative results. The chemical basis for any activity present seems still to await elucidation. Investigations are continuing into other compounds present in the leaves.

EXPERIMENTAL

Biological material. Fresh leaves of *Desfontainia spinosa* were obtained from the Younger Botanic Garden, Argyll, Scotland. The material was authenticated at source and a specimen voucher is deposited in the herbarium of the Department of Pharmacy, Chelsea College.

Isolation of iridoids. Dried and powdered leaves (150 g) were extracted with 500 ml of boiling water and, after standing for 15 min, the mixture was filtered to give a dark orange liquid with a bitter taste. Tests for alkaloids proved negative so the presence of iridoids was suspected. The filtrate was mixed with 100 g activated charcoal powder and filtered on a kieselguhr bed. The filtrate was colourless and had no bitter taste. The bed was eluted with H_2O (600 ml) until no free sugars could be detected in the filtrate. The bed was then eluted with 400 ml MeOH–H_2O (1:1). The eluate was concd under low pressure to give 445 mg of a pale green syrup which had a bitter taste. The syrup was dissolved in $CHCl_3$–MeCOEt (4:1) and fractionated by CC (silica gel 5 × 50 cm eluted with $CHCl_3$–MeCOEt mixtures in order of increasing polarity from 10:1 to 2:1). Fractions (25 ml) were collected, concd under red. pres. and analysed by TLC. Identical fractions were combined and individual compounds isolated by prep. TLC [silica gel GF_{254} 1 mm thick, solvents as below under TLC. Bands detected as quenching zones by examination under UV light (254 nm) were eluted with Me_2CO]. Three compounds were isolated and identified (as described above) as loganetin, loganin and loganic acid, being eluted in that order.

Loganin (40 mg) obtained as crystals (EtOH), mp 220°, was identical to that isolated by Bisset and Choudhury [3].

Loganic acid (90 mg) obtained as white crystals (MeOH), mp 186° hR_f (a) 0 (b) 5 (c) 20. Spectral features were identical to those published [4, 5].

Loganetin (82 mg) obtained as a white amorphous solid giving single spots in each TLC system used. hR_f (a) 45 (b) 85 (c) 95.

$[\alpha]_D^{20}$ −26° (MeOH). Spectral features were identical to those published [6, 7].

Detection of loganetin in fresh leaves Fresh leaves (20 g) were chopped and left to macerate in $CHCl_3$ for 2 hr at room temp. The extract was filtered and concd to dryness to give 760 mg residue. TLC of this residue showed the presence of a spot identical in colour and R_f values to that of loganetin.

TLC. Silica gel GF_{254}. Solvents: (a) $CHCl_3$–MeCOEt (4:1); (b) $CHCl_3$–MeOH (12:1); (c) MeCOEt–MeOH (12:1). Detection of zones was by examination under UV light (254 nm) after spraying with 0.5% anisaldehyde in HOAc–H_2SO_4–MeOH (2:1:17) and subsequent heating at 105° for 10 min. Loganin-like iridoids appear as quenching zones in UV light and as pink turning to red after visualization with the anisaldehyde reagent. Zones of other colours were detected and isolated and their identity is being established.

Tests for the presence of alkaloids. The following reagents (0.5 ml in each case) were added to 10 ml aliquots of the extract under investigation: Dragendorff's; Mayer's; Ehrlich's. The lack of formation of a precipitate with the first two reagents and the absence of a purple-blue colour after heating with the third was taken to indicate the absence of alkaloids in general and simple indole bases in particular respectively.

Hydrolysis of loganin. Loganin (75 mg) was dissolved in 10 ml H_2O, 10 mg emulsin added and the mixture incubated at 35° for 18 hr. After extraction with $CHCl_3$–MeOH (3:2) (2 × 20 ml) the organic layers were combined and concd. Prep. TLC (system a) yielded 30 mg loganetin giving identical spectral characteristics to those reported in the literature [6, 7].

Demethylation of loganin. Loganin (60 mg) was mixed with 20 ml 5% KOH in MeOH and refluxed for 15 min. The mixture was neutralized with methanolic HCl, filtered and the filtrate concd. Prep. TLC (system c) yielded 35 mg loganic acid with spectral characteristics identical to those in the literature [4, 5]. The same applied to the penta-acetate obtained from conventional acetylation of the loganic acid obtained [5].

Methylation of loganic acid. Loganic acid (20 mg) was methylated with CH_2N_2 using the Aldrich Mini Diazald kit. Prep. TLC (system b) on the reaction mixture yielded 12 mg of a product identical in chromatographic and spectral behaviour with loganin.

Acknowledgements—We wish to thank Mr. G. McDonough for ^1H NMR spectra and Mr. D. Carter and Mr. R. Harper for mass spectra. We thank Dr. N. G. Bisset for a generous sample of loganin. One of us (L.L.M.) is grateful to W.H.O. for a W.H.O. Fellowship.

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