M.A 243201.

The ABX system of the vinyl in **1** was solved with help of LAOCON III program. The ^{1}H NMR signals of **5** were: δ7.53 (*s*, H), 7.34 (*dd*, H, J = 8.9, J' = 1 Hz), 6.84–6.79 (*m*, 2H), 6.15 (*dd*, H, J = 10.5, J' = 17.4 Hz), 5.08 (*dd*, H, J = 10.5, J'' = 0.9 Hz), 5.07 (*dd*, H, J' = 17 4, J'' = 0 9 Hz), 3 86 (*s*, 3H) and 1 84 (*s*, 6H).

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Phytochemistry, Vol. 23, No 9, pp 2096–2097, 1984.
Printed in Great Britain

0031–9422/84 $3 00 + 0.00

METHYL *p*-COUMARATE: A CYTOTOXIC CONSTITUENT FROM *COMPTONIA PEREGRINA*

SHIRLEY N. HOOPER, TANNIS JURGENS, R. FRANK CHANDLER and MALCOLM F. G. STEVENS*

College of Pharmacy, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 3J5; *Department of Pharmacy, University of Aston, Birmingham, B4 7ET, U K.

(*Revised received* 19 *December* 1983)

Key Word Index—*Comptonia peregrina*, Myricaceae; sweet fern; cytotoxicity, toxicology; methyl *p*-coumarate.

Abstract—Methyl *p*-coumarate has been isolated from the roots and stems of *Comptonia peregrina*, a plant used by North American Indians, settlers and herbalists to treat a variety of skin conditions. Pharmacological testing has proven this compound to be cytotoxic.

INTRODUCTION

Among those screened as part of an ongoing study of medicinal plants used by the Maritime native peoples [1, 2] was *Comptonia peregrina* (L.) Coult. This shrub, commonly known as sweet fern, grows abundantly in poor soil in eastern North America. A literature search reveals that the only constituents characterized from this plant are essential oils [3] and flavonoids [4]. Indians of the Canadian Maritimes employed the leaves of *C. peregrina* in the treatment of sprains, swellings, and the inflammation caused by poison ivy [5]. Other North American Indians used this plant for a variety of dermatological problems as did the European settlers, who also used the plant for 'skin cancer' [4, 6]. Locally, a concoction of sweet fern roots has been reported of use against 'psoriasis' and 'eczema'.

RESULTS AND DISCUSSION

A phytochemical screening of *C. peregrina* indicated several components of possible medicinal interest [1, 2]. During the phytochemical analysis methyl *p*-coumarate was isolated in small amounts (< 0.0002%) from the ammonium hydroxide–methanol extract. Identification of the isolated compound was based upon chemical analysis, melting point, IR, ^{1}H NMR and ^{13}C NMR and GC/MS.

p-Coumaric acid, isolated first in 1865 by Hlasiwetz and synthesized in 1918 by Konek, is a well known and widely distributed plant constituent [7]. Methyl *p*-coumarate, however, has been reported only rarely as a natural product [8, 9] and there are few studies on biological effects. Among those effects reported are chlorophyll-degrading activity [10], marked inhibition of bacterial growth [11, 12] and possibily, coffee rust self-inhibition [13].

In vitro pharmacological testing of methyl *p*-coumarate against mouse TLX5 lymphoma cells demonstrated a significant inhibitory activity. The LD_{50} dose of the compound was 15 μg/ml. *In vivo* testing against P388 tumor indicated that the compound was inactive at doses of 400 mg/kg or less and toxic at doses of 800 mg/kg and greater. We believe this is the first report of cytotoxicity for this compound. Although nonselective, when administered topically, this compound may well account for the reported success of *C. peregrina* in the treatment of various dermatological problems. This is also the first report of methyl *p*-coumarate in the Myricaceae.

EXPERIMENTAL

C. peregrina was harvested in Nova Scotia in summer and air dried. Leaves were removed and the roots and stems ground in a Wiley mill (mesh size 5 mm) and extracted successively with petrol × 2, $CHCl_3$ × 3 and 10% NH_4OH in MeOH × 3. Methyl *p*-coumarate was obtained from the combined, partially reduced in volume, MeOH extracts by alternately partitioning between acid (HCl) and base (NH_4OH) with $CHCl_3$ as the organic solvent. Final recrystallization was from 5% HCl, mp 136–137° (lit 137° [14]). It was possible to monitor the isolation by GC of the $CHCl_3$ extract [9]. Methyl *p*-coumarate (Found C, 67 4; H, 5.6. Calc. for $C_{10}H_{10}O_3$, C, 67.4, H, 5 7%). EIMS (direct interface) 70 eV, *m/z* 178 $[M]^+$ ($C_{10}H_{10}O_3$), 147 $[M-OMe]^+$ and 119 $[M-CO_2Me]^+$ [15]. Spectra were in agreement with

those published elsewhere [16, 17]. The ^{1}H NMR spectrum, recorded at 80 MHz for a $CDCl_3$ soln showed the methoxyl CH_3 at δ3.77 (*s*, 3H), the AB pattern for the *trans* substituted double bond (J = 16 Hz) at δ6.27 (*d*, 1H) and 6.82 (*d*, 1H), and the AA'BB' multiplet for a *para* substituted aromatic ring at δ6 82 and 7.40 The phenolic proton was not observed in this soln, but was observed at δ3.70 for a $CDCl_3$ soln recorded at 250 MHz. The ^{13}C spectrum, recorded at 20 MHz for a $CDCl_3$ soln, showed the methyl carbon at δ51 6, the olefinic carbons at δ114.8 and 144.9, the phenyl C-1 at δ144.4, the phenyl C-4 at δ158.1, and the remaining aromatic carbons at δ129.9 (2C) and δ115.8 (2C) and the carbonyl carbon at δ168.1.

Subsequent to the initial isolation, an extraction with boiling water was carried out on a further batch of plant material. Methyl *p*-coumarate was again found, thus eliminating the possibility that the ester was an artifact of the MeOH extraction.

The *in vitro* testing was conducted against a culture of mouse TLX5 cells growing in Dulbecco's modified Eagle's medium containing 4 5 mM glucose. The cells were grown in wells in a total volume of 1 ml of media/drug soln. Solns were inoculated with lymphoma cells (initial count 6.19×10^4) and then the cells were counted at day 4 with a Coulter Counter.

The *in vivo* testing was conducted against the P388 tumor (10^6 cells) implanted i.p. in BDF^1 mice. Methyl *p*-coumarate dissolved in 10% DMSO in peanut oil was administered i.p for 5 days The percentage increase in survival time relative to controls which received solvent only was recorded. At doses of 400 mg/kg/day, or less, there was no increase in survival time in the treated group, however at 800 mg/kg/day the drug proved to be toxic as evidenced by excessive weight loss in the treated group

Acknowledgements—We thank Drs W. D Jamieson and E Lewis, Atlantic Research Laboratory, National Research Council, Halifax, Nova Scotia, Canada, for GC/MS spectra; Dr D. L Hooper, Department of Chemistry, Dalhousie University, Halifax, Nova Scotia and Drs. M. Smith and C. Rodger, Bruker Spectrospin Canada, Ltd , Mississauga, Ontario Canada (^{1}H, 250 MHz) for NMR spectra We are also grateful to Mr. A. Wilson, Curator of Botany, Nova Scotia Museum, Halifax for identification of the plant material and Ms. R. Brennan for conducting the cytotoxicity test against TLX5 lymphoma cells *in vitro*. This work was supported by the Medical Research Council of Canada (MA-6448).

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