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*Phytochemistry,* 1980, Vol. 19, pp. 704–705. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0401-0704$02.00/0

# *N*-FERULOYLGLYCYL-L-PHENYLALANINE ISOLATED BY PARTIAL HYDROLYSIS OF BULK LEAF PROTEIN OF LUCERNE, *MEDICAGO SATIVA*, CV EUROPE

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(*Revised received* 30 *July* 1979)

**Key Word Index**—*Medicago sativa*; Leguminosae; bulk leaf protein; *p*-coumaric acid; ferulic acid; *N*-feruloylglycine; *N*-feruloylglycyl-L-phenylalanine.

Phenolic acids most frequently occur bound as esters, glycosides or amides [1–5]. In addition, a pseudopeptide-type of bonding, in which the carboxylic group of ferulic acid is attached to the amino function of the *N*-terminal amino acid of a polypeptide chain, has been described. This is *N*-feruloylglycyl-L-phenylalanine which was found as a terminal sequence of a barley globulin fraction [1]. The same sequence has now also been recognized in lucerne bulk leaf proteins and this paper describes briefly its identification from this new source.

## EXPERIMENTAL

Lucerne bulk leaf proteins were obtained from R. L. M. Synge (Norwich, U.K.). They were extracted with PhOH–HOAc–$H_2O$ (1:1:1) as described [6]. The extract was directly concd, the phenol removed and the concentrate slurried in water and freeze-dried. Before analysis this undialysed protein fraction was first thoroughly extracted with $Et_2O$ in order to remove any remaining free phenolic acids. Thereafter the residue was treated, under $N_2$, with boiling 2 N NaOH (2 hr). This treatment mainly releases the ester-bound phenolic acids, which, after acidification and subsequent $Et_2O$ extraction, were analysed as described [7]. These analyses showed the presence of 5.40 mg alkali-labile bound ferulic acid and 1.53 mg alkali-labile bound *p*-coumaric acid per g protein material. However, as was shown by pilot experiments synthetic *N*-feruloylglycyl-L-phenylalanine (FGP) [8, 9] (2.5 μmol), proved under the same conditions, to be completely hydrolysed (products formed: *N*-feruloylglycine (FG) [8, 10] (1.25 μmol), ferulic acid (1.25 μmol), glycine (1.25 μmol) and phenylalanine (2.5μmol)). Thus the foregoing alkaline treatment may also result in a partial release of peptide-bound ferulic acid from protein. After alkaline hydrolysis, the remaining protein material was further repeatedly treated (×16) for 15 min periods with boiling 4 N HCl in $N_2$. Successive partial hydrolyses are required because the ferulic acid-containing peptides, which are formed during hydrolysis, are continuously and rapidly split by boiling acids. Furthermore, liberated ferulic acid is also rapidly converted into several decomposition products [11]. After each hydrolysis, the hydrolysate was cooled and the released peptides and remaining free phenolic acids were thoroughly extracted with EtOAc. These extracts were then pooled from 4 consecutive hydrolyses, washed with $H_2O$ and concd *in vacuo* to a small vol. The resulting concentrates were diluted with 2 ml EtOH and chromatographed by PLC with toluene–$HCO_2Et$–$HCO_2H$ (5:4:1) on Si gel-cellulose MN (1:1) mixtures [7, 12, 13]. The spot corresponding to the synthetic FGP standard was eluted with EtOH (×6) and then further purified by means of METC (Multiple elimination TLC) [1, 7, 14, 15]. 0.25% $NH_4OH$, MeOH–$H_2O$ (3:1) (with steaming), *sec*-BuOH–$H_2O$ (4:1) and toluene–$HCO_2Et$–$HCO_2H$ (5:4:1) (with steaming) were used in sequence. Finally the purified material, showing the same $R_f$ and blue fluorescence (greenish after spraying with 2 N NaOH) as the standard, was removed from the plate and eluted with 0.1 N NaOH. Comparison of the UV spectra, the excitation and fluorescence maxima and the $R_f$ values in four solvents proved the identity of the unknown with synthetic FGP. The combined extract corresponding with the 3rd-hour hydrolysis of lucerne protein contained the highest amount of *N*-feruloylglycyl-L-phenylalanine. Further support for the presence of amino acid-bound ferulic acid in lucerne proteins was obtained by the subsequent isolation of *N*-feruloylglycine

(FG) from the partial hydrolysates. This was purified, compared with synthetic material and identified by similar procedures as for FGP.

*Acknowledgements*—The authors gratefully acknowledge the support of their work by the E.E.C., the F.K.F.O. and the I.W.O.N.L.

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*Phytochemistry*, 1980, Vol. 19, pp. 705–707. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0401-0705$02.00/0

# 13-OXOMYRICANOL, A NEW [7.0]-METACYCLOPHANE FROM *MYRICA NAGI*

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(*Revised received* 12 *September* 1979)

**Key Word Index**—*Myrica nagi*; Myricaceae; diarylheptanoid; [7.0]-metacyclophane; 13-oxomyricanol.

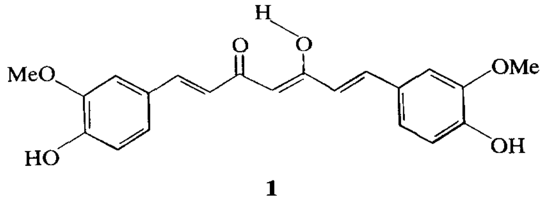
**Abstract**—A new [7.0]-metacyclophane has been isolated from the root bark of *Myrica nagi*. On the basis of spectroscopy the constitution of the new compound is proposed to be 13-oxomyricanol.

## INTRODUCTION

Diarylheptanoids comprise a class of natural products based on 1,7-diphenylheptane. They are rare, but *ca* 40 substances are known from one family in the monocotyledons (Zingiberaceae) and four in the dicotyledons, of which the Betulaceae and the Myricaceae are richest in these substances. Most diarylheptanoids are acyclic, with curcumin (**1**), the pigment of *Curcuma longa* rhizomes [1], as an example.

Some diarylheptanoids with a skeletal structure based on diphenyl ([7.0]-metacyclophanes) or diphenyl oxide (14-oxa-[7.1]-metaparacyclophanes) are known. Both types are constituents in the genus *Myrica*: myricanol (**2**) and myricanone (**3**), isolated from *M. nagi* [2], and porson (**4**) from *M. gale* [3] are examples of the diphenyl type, while galeon (**5**) and hydroxygaleon (**6**), both from *M. gale* [4], are of the diphenyl oxide type.

In this communication we wish to report the isolation of a new [7.0]-metacyclophane from *Myrica nagi*. Its constitution was established as 13-oxomyricanol (**7**) on the basis of spectroscopic evidence.



**1**