Food Chemistry **40** (1991) 113–117

Short Communication

Identification of Bovolide and Dihydrobovolide in Beef

ABSTRACT

Bovolide, a compound first isolated as a new γ-lactone from butter and so-named because of its bovine origin, was identified here, together with dihydrobovolide, for the first time from cooked beef. The compounds were isolated by simultaneous steam distillation/solvent extraction and identified by combined capillary gas chromatography/mass spectrometry.

INTRODUCTION

Bovolide (**1**; 2,3-dimethyl-4-hydroxynona-2,4-dienoic acid, lactone or 2,3-dimethylnona-2,4-dien-4-olide) was first isolated and identified as a new γ-lactone from butter in the 1960s (Boldingh & Taylor, 1962; Lardelli *et al.*, 1966), and was so-named because of its bovine origin. Despite this fact, it has never been reported from beef. This short communication describes its first identification—together with dihydrobovolide (**2**; 2,3-dimethyl-4-hydroxynon-2-enoic acid, lactone or 2,3-dimethylnon-2-en-4-olide)—from cooked beef.

MATERIALS AND METHODS

Fresh fillet steak, purchased from a local butcher, was trimmed of all excess fat and minced to a particle diameter of 4 mm.

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Isolation of volatile components

The minced beef (1 kg), in distilled water (80 ml), was extracted for 4 h in a modified (MacLeod & Cave, 1975) Likens and Nickerson (1964) apparatus using triply-distilled dichloromethane (50 ml). Five extractions were performed and the extracts combined before concentration to 500 μl using a rotary evaporator (20°; 3×10^2 N m^{-2}). A blank isolate was obtained as above, using distilled water only in the extraction flask.

Capillary gas chromatography

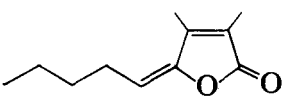
A fused silica bonded-phase capillary column was used under the following conditions: 60 m × 0·32 mm i.d. DB5 column (1 μm film); helium, 2 ml/min; temperature programme, 50° increased at 2°/min to 225°; detector (fid) and injector heaters, 225°; injection volume, typically 1 μl at 20:1 split and attenuation, 1×64, i.e. 32×10^{-11} A fsd.

Combined capillary gas chromatography/mass spectrometry

A Kratos MS890 instrument was used, linked on-line to a Kratos DS90 data processing system. Capillary GC conditions as above were used. Significant operating parameters of the MS were: ionization voltage, 70 eV; ionization current, 100 μA; source temperature, 200°; accelerating voltage, 8 kV; resolution, 1000; scan speed, 1 s/decade (repetitive throughout run).

RESULTS AND DISCUSSION

Using established procedures, volatile components were isolated from fresh lean fillet steak, and analysed by combined capillary GC/MS. Two of the identified components, present in trace amounts, were bovolide (**1**; I = 1543) and dihydrobovolide (**2**; I = 1536).



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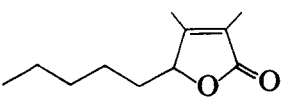

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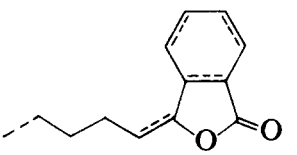
The recorded mass spectra (**1**: *m/z* 124(100) 137(92) 55(74) 27(55) 39(35) 54(34) 29(29) 81(29) 180(M, 28) 43(24) 125(20) 96(18) 82(15); **2**: *m/z* 83(100) 55(54) 111(42) 43(40) 41(38) 112(32) 27(27) 39(24) 29(23) 182(M, 22) 69(17) 53(16) 99(12) 153(8)) are in excellent agreement with reference mass spectra (Lardelli *et al.*, 1966; Sakata & Hashizume, 1973).

Both bovolide and dihydrobovolide have been reported previously from tobacco (e.g. Demole & Berthet, 1972; Lloyd *et al.*, 1976; Corbier *et al.*, 1988), tea (Horita *et al.*, 1985), peppermint oil (Sakata & Hashizume, 1973; Takahashi *et al.*, 1980; Yoshida *et al.*, 1984) and the leaves of *Lycium chinese* (Sannai *et al.*, 1983) and *Carphephorus corymbosus* (Karlsson *et al.*, 1972). In addition, bovolide itself has been rather more widely reported in a range of different teas (e.g. Kawakami *et al.*, 1986, 1987, 1989; Owuor *et al.*, 1987), in textured soy protein (Ames & MacLeod, 1984) and in the leaves of *Gardenia jasminoides* and *Magnolia grandflora* (Horita *et al.*, 1985).

Both compounds possess strong odours described as celery-like (Boldingh & Taylor, 1962; Lardelli *et al.*, 1966) and indeed, have been patented for imparting such an aroma to improve the flavour of tobacco (Schumacher & Roberts, 1966). The close structural resemblance that they share with compounds of general formula **3** (i.e. phthalides), which have been synthesised (Lardelli *et al.*, 1966) and identified as character impact components of celery (e.g. MacLeod & Ames, 1989), is an interesting example of structure–activity correlation.



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Although both components therefore contribute to cooked beef aroma, they are not character impact compounds of beef flavour.

Bearing in mind that bovolide was so-named because of its bovine origin, it is a little surprising that it (and its dihydro counterpart) has not been reported previously among the approximately 800 volatile components of heated beef. The original authors who isolated and characterised it as a new γ-lactone from butter have suggested that, in the absence of other homologues, it is not connected with normal biological fatty acid synthesis (Boldingh & Taylor, 1962; Lardelli *et al.*, 1966). They also remarked that it should not be regarded as a contaminant, since it was present in butters of widely different provenance, including ghees, the Asiatic melted butterfats. They showed that its origin in butter is from grass in the cow fodder. The same origin would explain its presence in beef.

ACKNOWLEDGEMENT

Thanks are due to Mr W. G. Gunn and Mr A. E. Cakebread for operating the GC/MS.

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(Received 7 February 1990; revised version received and accepted 27 April 1990)