Phytochemistry, 1976, Vol. 15, pp. 1713–1715. Pergamon Press. Printed in England.

BAKKENOLIDE-A. ITS DISTRIBUTION IN *PETASITES* SPECIES AND CYTOTOXIC PROPERTIES

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(*Received* 24 *March* 1976)

Key Word Index—*Petasites*; Compositae; fatty acids, bakkenolide-A, chemotaxonomy, cytotoxicity, transformed cells.

Abstract—A rapid GLC method is described for the determination of bakkenolide-A in small amounts of plant material. The distribution of bakkenolide-A among the different lipid classes of *Petasites albus* is discussed and also the distribution among different parts of the plant during a growing season. Smaller amounts of bakkenolide-A are found in *P. hybridus* buds and only trace amounts in *P. fragrans*.

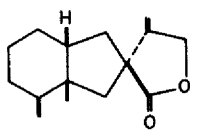
Cytotoxicity tests showed bakkenolide-A has marked cytotoxic activity and potential anti-tumour properties.

INTRODUCTION

A great many sesquiterpenes with an eremophilane skeleton have been isolated from *Petasites* species [1,2] and the distribution of these compounds has been used in attempts to solve the taxonomic problems of the most common European species [3,4]. The main sesquiterpene lactone present in *P. albus* and *P. japonicus* is bakkenolide-A (fukinanolide) (1) and the structure of this compound has been elucidated by various Japanese investigators [5,6].

The present paper reports the development of a rapid GLC method for the determination of bakkenolide-A (1) in relatively small amounts of plant material. The method was used to investigate the distribution of bakkenolide-A (1) in various *Petasites* species growing in the west of Scotland and to determine the changes in amounts during the growing season of *P. albus*.

Also, in the present work, the cytotoxic properties of bakkenolide-A (1) a β-methylene-γ-lactone, were compared with those of α-methylene-γ-lactones from other Compositae species.



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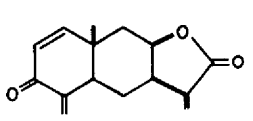

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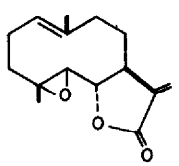

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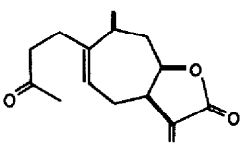

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RESULTS AND DISCUSSION

During the GLC investigation of the methyl esters from the total leaf lipids a peak, representing about 10% of the total peak area, was found in a region of the chromatogram which usually showed only trace components when methyl esters from angiosperm leaves were being examined. This peak was also found in the chromatograms of the methyl esters from the buds, scale leaves and flower heads and could represent up to 60% of the total peak area. After separation of the leaf lipids into different classes this peak was only found in the neutral lipids where it accounted for 45% of the total peak area. The compound giving rise to this peak was separated by PLC of the methyl esters and was shown to be bakkenolide-A (1).

When the total leaf lipids were examined by GLC using polyester columns with the same operating conditions as for methyl esters the only major peak obtained was that for bakkenolide-A (1). The variation of retention of bakkenolide-A (b-A) with column polarity could be represented by $ECL_{b\text{-}A} = 1.88 \times ECL_{18:3\ n\text{-}3} - 16.48$ [7,8]. The 'slope value' of 1.88 was higher than that of methylene-interrupted hexaenoic methyl esters. This GLC method could be used to determine the amount of bakkenolide-A (1) in small amounts of plant material when methyl eicosanoate was added as an internal standard.

The amounts of bakkenolide-A in various parts of *P. albus* during a growing season are shown in Fig. 1. There were large increases in the amounts of bakkenolide-A in the scale leaves and flowers during the growing season, maxima occurring when the flowers were at maturity. The amount present in the ordinary leaves remained constant during that part of the growing season after the flower stalks had withered.

The average amounts of bakkenolide-A (1) in the buds of *P. albus* from two sites, *P. hybridus* from three sites,

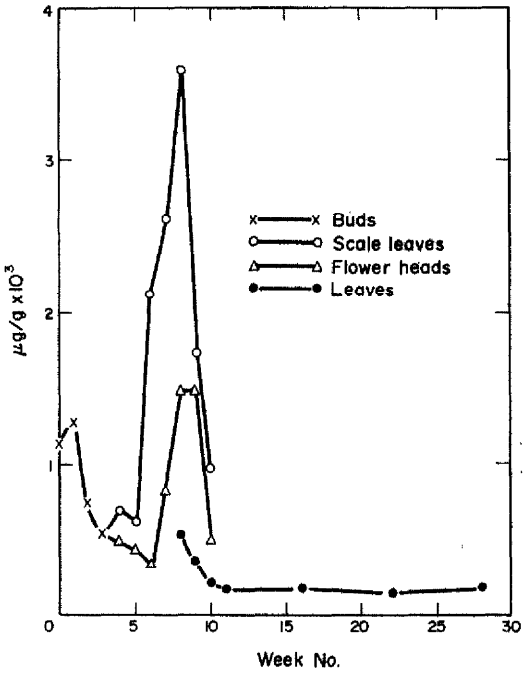

Fig. 1.

and *P. fragrans* from one site are shown in Table 1. The greatest amounts were found in the scale leaves of *P. albus* and there were similar amounts in the buds from the two different sites. Shirahata *et al.* [5] found 400 μg/g in fresh buds of *P. japonicus* which is 2.5–3 times less than in our samples of *P. albus*. The amounts in our three samples of *P. hybridus* buds were similar and were 2.5×10^{-2} times less than in the *P. albus* samples. Keates *et al.* [9] found 6 μg/g in *P. hybridus* leaves gathered from a site in Central Scotland. Novotny *et al.* [4] found that substances of the bakkenolide type were completely absent in *P. hybridus* and *P. kablikianus*. There were only traces of bakkenolide-A in our sample of *P. fragrans*.

The results obtained from cytotoxicity studies on a number of normal and transformed cells derived from different organisms are summarised in Table 2. All lines of human origin were markedly more sensitive than cells of rodent origin whether normal or transformed. However human cell lines derived from human carcinomas (H.Ep.2 and HeLa) exhibited an ED_{50} of *ca* 1 μg/ml, while the control 'nontransformed' cell line (HeLu) was resistant to a five-fold higher concentration.

The results also show that rat cells which have been transformed by HSV-2 (Rel cells) [10] were 2.25 times more sensitive than the control rat cell line (Hood). BHK21/C13 cells, which are a continuous cell line of hamster origin, showed an ED_{50} similar to that of RE1 cells.

The fact that bakkenolide-A was relatively more toxic to cell lines which are either tumour derived or transformed should be viewed with caution because of the vast range of ED_{50} values found on normal cells derived from different host organisms, however, the results are indicative of a possible anti-tumour role for bakkenolide-A. Further studies are underway to determine the effects of this compound on a wider range of transformed cells.

Many sesquiterpene lactones from the Compositae family have been found to have cytotoxic activity [11–13]. From a study of the chemical structures it was noted that those lactones with the highest cytotoxicity were α,β-unsaturated and that the α,β-olefinic group was exocyclic [12]. It has been suggested [11] that although the α-methylene-γ-lactone moiety confers high cytotoxic activity, the necessity is for the $O{=}C{-}C{=}CH_2$ system, whether it be in a lactone or a ketone. Bakkenolide-A (**1**) is a β-methylene-γ-lactone and does not have a $O{=}C{-}C{=}CH_2$ system, but it gives a similar ED_{50}(H.Ep.2) value to that of encilin (**2**), parthenolide (**3**) and xanthinin (**4**), all of which have the α-methylene group [11].

Table 1. Amounts (μg/g fr. wt) of bakkenolide-A in *Petasites* species

Species	Site	Buds	Scale leaves	Flower heads	Leaves
P. albus	1	1030	1730	770	210
	2	1260	—	—	—
P. hybridus	3	33	—	—	—
	4	27	—	—	—
	5	30	—	—	—
P. fragrans	6	less than 1	—	—	—

Table 2. Cytotoxicity of bakkenolide-A

Cell line	ED_{50} (μg/ml)
HEp2	0 8
HeLa	1
HeLu	5
RE 1	18
BHK 21/C13	20
Hood	40

HEp2—epidermoid cell line derived from a carcinoma of the larynx; HeLa—epitheloid cell line derived from a carcinoma of the cervix; HeLu—normal human embryo lung fibroblastic cells; RE 1—ref. [10]; BHK 21/C13—hamster embryo fibroblastic cells; Hood—rat embryo fibroblastic cells.

EXPERIMENTAL

Samples of *Petasites* species were collected from various locations in North Ayrshire, Scotland. Lipids were extracted and separated into classes by methods described previously [14]. GLC analyses of the total lipid methyl esters and the methyl esters from each lipid class were carried out on a PE800 chromatograph using open tubular columns of different polarity [7,8]. TLC was carried out on 0.25 mm and 0.75 mm layers of Si gel in glass tanks lined with filter paper and hexane–Et_2O (80:20) as eluting solvent. After spraying with ammonium bisulphate and heating to 150°, bakkenolide-A gave a characteristic dark purple coloured spot. PLC of the total lipid extract was used to obtain bakkenolide-A and the impure material from this separation was recrystallised from aq. MeOH to give white crystals mp 79–80° (80–81° ref. 5); (found C 77.01, H 9.46, M^+ 234; $C_{15}H_{22}O_2$, mol. wt. 234.33, requires C 76.92, H 9.40%). The MS and IR were identical to published spectra [5]. BHK21/C13 [15], HEp2 [16], HeLa [17] and HeLu [18] cells are all continuous cell lines regularly grown in Eagle's medium supplemented with 10% calf serum (EC 10). Hood cells [10] are a normal rat embryo fibroblastic cell line and RE 1, a rat fibroblastic cell line transformed by a temperature sensitive mutant of *Herpes simplex*, type 2 (HSV-2) [10] grown in Eagle's medium containing 10% foetal

calf serum (EFc10). Cell suspensions were seeded at a concentration of 10^5 cells/ml in microtitre plates to give a final cell concentration of 5×10^4 cells per well in a total vol of 1 ml EC10 or EFc10, and incubated in a humidified CO_2 atmosphere at 37°. Bakkenolide-A, dissolved at a concentration of 10 mg/ml in 50% DMSO and diluted to the desired concentration in growth medium, was substituted for the original medium 12 hours post seeding and the plates incubated at 37° until the control cells (either no addition or DMSO alone) became confluent (seven days). The cells were then fixed and stained with Geimsa Blue and, after drying, the cell density was determined using a Joyce Loebel densitometer. The % surviving cell fraction was calculated using the average reading of twelve wells per determination against both untreated control cells and cells treated only with DMSO.

Acknowledgement—Thanks are due to Mrs. Joan MacNab for providing the RE 1 and HOOD cells.

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