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# Psoriatic skin lesions contain a novel lipid neutrophil chemokinetic compound which is distinct from known chemoattractant eicosanoids

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**1** Lipid extracts of scale from the lesions of the skin disease psoriasis were purified by high performance liquid chromatography (h.p.l.c.). Assay of fractions by an agarose microdroplet method showed the presence of a novel neutrophil chemokinetic compound which possessed the chromatographic properties of a monohydroxy fatty acid, yet was distinct from the chemoattractant eicosanoid, 12-hydroxyeicosatetraenoic acid, previously isolated in psoriasis.

**2** The novel material, termed compound X, was also detected in fractions collected on h.p.l.c. of extracts of chamber fluid samples obtained from abraded psoriatic lesions, but was not detectable in samples from clinically normal skin.

**3** Comparison of the straight and reversed phase h.p.l.c. retention times of compound X with those of a range of standard monohydroxy fatty acids, together with further analysis by gas chromatography – mass spectrometry and assay of selected standards for neutrophil chemokinetic activity, failed to reveal the structural identity of compound X.

**4** The finding of a further compound in psoriatic lesions, which stimulates neutrophil movement, highlights the complexity of inflammatory mediator production in this disease.

## Introduction

Lipid extracts of samples from the lesions of the common inflammatory skin disease, psoriasis, have been shown to contain neutrophil chemoattractant eicosanoids, including a leukotriene $B_4$ ($LTB_4$)-like compound (Brain *et al.*, 1984a,b) and 12-hydroxyeicosatetraenoic acid (12-HETE) (Hammarström *et al.*, 1975; Camp *et al.*, 1983; Barr *et al.*, 1984), a significant portion of the latter material consisting of the novel 12(**R**) isomer (Woollard, 1986; Cunningham & Woollard, 1987). These compounds, together with other chemoattractants which have been identified in lesional extracts and which include platelet-activating factor (Mallet *et al.*, 1984; Mallet & Cunningham, 1985), C5a des Arg (Takematsu *et al.*, 1986; Schröder & Christophers, 1986) and partially characterized 10–30 kD material (Camp *et al.*, 1986; Schröder & Christophers, 1986), may play a role in eliciting the neutrophil infiltrates (Ragaz & Ackerman, 1979) that characterize psoriasis.

We have now demonstrated the presence of a further, previously undescribed chemoattractant compound in lipid extracts of samples from psoriatic skin lesions.

## Methods

### *Patients*

All volunteers gave informed consent and ethical committee approval was obtained. Samples were recovered from the skin lesions of adult male and female patients with chronic plaque psoriasis who had received no treatment for at least one week. Scale was obtained by gentle abrasion of the surface of lesions with a scalpel blade and the material stored at −20°C before analysis. The lesions and clinically normal skin of patients were also sampled

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by using a skin chamber technique, as described previously (Brain *et al.*, 1984b). Briefly, two lesional areas and two areas of clinically normal, control skin were scraped with a scalpel blade to yield superficial, circular abrasions with minimal punctate bleeding, measuring approximately 22–24 mm in diameter. Acrylic cylinders (internal diameter 19 mm) were fixed to the abrasions with cyanoacrylate adhesive such that fluid added to the chambers was in contact only with abraded skin. Each site was then washed with sterile phosphate buffered saline (PBS, 1 ml) for 5 min, the washings discarded, and a further 1 ml sterile PBS added to each chamber. After 30 min the PBS was removed, duplicate samples pooled and stored at −20°C.

*Sample extraction*

Scale (75–500 mg) was weighed, then homogenised in a mixture of ethyl acetate and 0.1 M sodium acetate buffer, pH 3.5 (6 ml each) by use of a motor-driven Teflon pestle in a glass homogeniser. Following centrifugation, the organic phase was removed, and the aqueous phase repartitioned twice with 6 ml ethyl acetate. The pooled organic phases were evaporated, vacuum desiccated and the residue partitioned between 4 ml n-heptane and 3 ml methanol, to remove non-polar material. The upper phase was discarded, the methanolic layer evaporated, and the residue stored in 50 $\mu$l methanol at −20°C.

Chamber fluid was mixed with 2 ml 0.1 M sodium acetate buffer, pH 3.5, and partitioned twice with 4 ml ethyl acetate. Following centrifugation, the pooled organic phases were evaporated and the residues partitioned between n-heptane and methanol as described for the scale samples.

*Chromatography*

Scale and chamber fluid residues, redissolved in 0.1 ml h.p.l.c. solvent, were purified by straight phase h.p.l.c. by use of two 25 cm × 4.9 mm silica columns (Nucleosil 50–5 $\mu$m and Spherisorb S5W, both from Hichrom, Reading, U.K.) connected in series and eluted with hexane/propan-2-ol/acetic acid (96 : 4 : 0.1 v/v) at 1 ml $min^{-1}$. One min fractions were collected, evaporated, vacuum desiccated and stored in 50 $\mu$l methanol at −20°C. Selected straight phase h.p.l.c. fractions were repurified by reversed phase h.p.l.c., by use of a 25 cm × 4.9 mm Spherisorb S5ODS column (Hichrom, Reading) eluted with methanol/water/acetic acid (80 : 20 : 0.01 v/v) at 1 ml $min^{-1}$. One minute fractions were collected, evaporated and stored as described above for the straight phase h.p.l.c. fractions.

*Biological assay*

Stored chromatography fractions and standard material were vacuum desiccated to remove methanol and the residues redissolved in minimal essential medium (MEM) buffered to pH 7.4 with 30 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) buffer. The neutrophil chemokinetic activity of each redissolved sample was determined in an agarose microdroplet chemokinesis assay as described previously (Smith & Walker, 1980; Camp *et al.*, 1983), activity being expressed as chemokinetic movement (mm) after subtraction of random movement in the presence of HEPES-buffered MEM alone. The mean random movement measured in a series of 20 assays carried out during the present study was 0.52 ± 0.09 mm (s.d.).

*Chromatography standards*

Racemic monohydroxy fatty acid standards were synthesized by photo-oxidation of eight commercially available $\omega$-3 or $\omega$-6 polyunsaturated fatty acids, followed by sodium borohydride reduction and purification by Lipidex 5000 chromatography and straight phase h.p.l.c., as described previously (Camp *et al.*, 1983). Racemic 12-hydroperoxyeicosatetraenoic acid (12-HPETE) was synthesized from arachidonic acid by the same photo-oxidation and purification procedures, but omitting the sodium borohydride reduction step. 12(R)-HETE was prepared as previously described (Woollard, 1986). All standards were stored in methanol at −20°C. The retention times of selected standards were determined in the two h.p.l.c. systems described, by monitoring column effluent for ultraviolet absorbance at 236 nm. Certain of these compounds were also tested for activity in the agarose microdroplet chemokinesis assay and were used as standards in gas chromatography–mass spectrometry (g.c.m.s.) assays.

*Gas chromatography–mass spectrometry*

Selected fractions obtained after successive straight and reversed phase h.p.l.c. purification of psoriatic scale samples (225 mg each) were reacted successively with diazomethane dissolved in diethyl ether/methanol (10 : 1 v/v) and with *bis*-trimethylsilyltrifluoroacetamide (BSTFA), to yield methyl ester, trimethylsilyl ether (MeTMS) derivatives. These derivatives were analysed by on-column injection on a 12 m × 0.3 mm SE30 (PhaseSep, Queensferry) silica g.c. column, temperature programmed from 175 to 280°C and interfaced with a VG 305 mass spectrometer. Electron impact ionisation at 40 ev was used

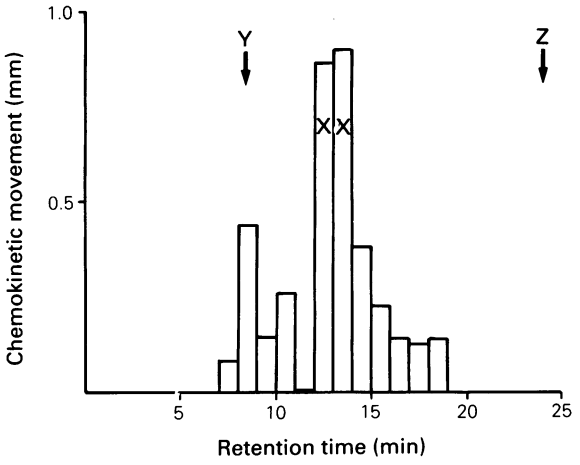


**Figure 1** Chemokinetic activity in an extract of psoriatic scale after purification by straight phase h.p.l.c. Collection of the first h.p.l.c. fraction began 7 min after injection of the material to be purified. One third of each fraction was evaporated, redissolved in 0.3 ml HEPES-buffered MEM, and assayed for chemokinetic activity without further dilution. Activity has been expressed as the distance moved by leucocytes less random movement in the presence of HEPES-buffered MEM alone. The mean of duplicate observations is given for each h.p.l.c. fraction. The elution times of standard 12-hydroxyeicosatetraenoic acid (12-HETE, Y) and 5-HETE (Z) are shown. The remaining two thirds of selected fractions (X) were repurified by reversed phase h.p.l.c. (Figure 2).

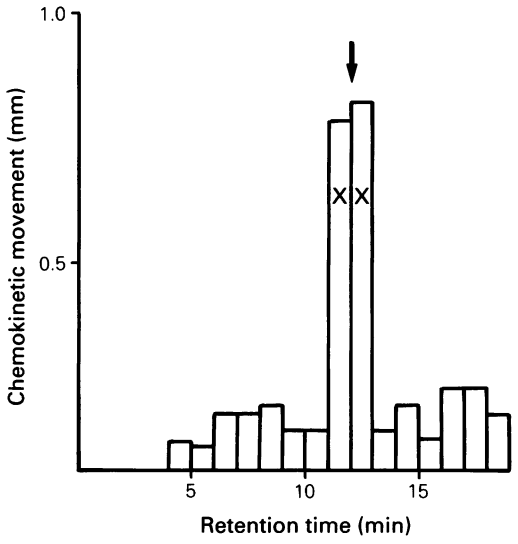


**Figure 2** Chemokinetic activity in reversed phase h.p.l.c. fractions after preliminary purification of compound X in a psoriatic scale extract by straight phase h.p.l.c. Collection of the first reversed phase h.p.l.c. fraction began 4 min after injection. Each fraction was halved, evaporated, one half redissolved in 0.3 ml HEPES-buffered MEM and assayed for chemokinetic activity without further dilution. Activity has been expressed as the distance moved by leucocytes less random movement in the presence of HEPES-buffered MEM alone. The mean of duplicate observations is given for each h.p.l.c. fraction. The arrow shows the elution time of standard 12-hydroxyeicosatetraenoic acid (12-HETE). The remaining halves of selected fractions (X) were subjected to analysis by g.c.m.s., as described.

and data recorded on a VG 2025 data system. Identification of the selected hydroxy fatty acids was carried out by comparison with the retention times of standards and monitoring of characteristic m/z values.

*Materials*

Spectrophotometric grade n-heptane and N-methyl-N-nitroso-*p*-toluenesulphonamide (used for generation of diazomethane) were obtained from Aldrich, Gillingham. All other solvents were of h.p.l.c. grade and obtained from Fisons, Loughborough. Materials used in the agarose microdroplet chemokinesis assay were obtained as previously described (Camp *et al.*, 1983) except for agarose (Uniscience, London). BSTFA was obtained from Sigma, Poole, and all other reagents from B.D.H., Poole.

## Results

Figure 1 shows the profile of biological activity obtained after purification of an extract of psoriatic scale (225 mg) by straight phase h.p.l.c. and assay of 1 min fractions (7–19 min) for chemokinetic activity. Increased chemokinetic activity was apparent in two sets of fractions, the first set (8–9 min, Figure 1) co-eluting with standard 12-HETE. The second set (12–14 min, Figure 1) contained material, henceforth termed compound X, which consistently caused greater chemokinetic activity than that co-eluting with 12-HETE. The same pattern of two peaks of chemokinetic activity was seen in a total of ten experiments with scale samples (75–225 mg) from different patients. No other consistent peaks of chemokinetic activity were seen in the ten experiments, including three in which fractions were collected from 4 min (representing the void volume of the column system) until after the elution time of 5-HETE (26–28 min). The retention time of a 12-HETE standard, determined in ten straight phase h.p.l.c. experiments, was between 8–9 min. In these experiments the fractions containing compound X

were found to elute between 3–6 min after the retention time of 12-HETE.

In four experiments, including that illustrated in Figure 1, psoriatic scale samples (225 mg each) were extracted, purified by straight phase h.p.l.c., and one third of each one min fraction assayed for chemokinetic activity. In each case, the remaining two-thirds of the two fractions containing compound X were pooled, re-purified by reversed phase h.p.l.c., and half of each fraction assayed for chemokinetic activity. The profile of activity shown in Figure 2 was obtained in all four experiments, and demonstrates that compound X co-elutes with standard 12-HETE in this h.p.l.c. system. In each experiment, the remaining halves of the two reversed phase h.p.l.c. fractions containing compound X were pooled, derivatised as described and analysed by g.c.m.s.

Lesional chamber fluid samples (2 ml) were obtained from five patients, extracted and purified by straight phase h.p.l.c. as described above. Assay of evaporated fractions again showed chemokinetic activity eluting as a discrete peak 3–4 min after the 12–HETE standard in all samples. Chamber fluid samples were also obtained from the clinically normal skin of three of the five patients. Assay of the samples from normal skin after straight phase h.p.l.c. purification showed little or no chemokinetic activity due to compound X. Results obtained on analysis of chamber fluid from the lesional and clinically normal skin of a single volunteer are shown in Figure 3. In two of the five lesional chamber fluid samples, biologically active amounts of 12-HETE were not detectable, one such result being illustrated in Figure 3.

In a single experiment, bulked psoriatic scale (500 mg) was extracted and purified by straight phase h.p.l.c. as described above. One quarter of each fraction was assayed by the agarose microdroplet method and a profile of activity similar to that seen in Figure 1 was obtained (Figure 4a). The remaining material in the three fractions containing compound X was redissolved in 0.9 ml HEPES-buffered MEM and assayed for chemokinetic activity in doubling dilutions. The chemokinetic activity of racemic 12-HETE and 12(R)-HETE was determined in the same assay (Figure 4b). The slopes of the dose-response curves for racemic 12–HETE, 12(R)-HETE and compound X were similar (Figure 4b).

As the chromatographic mobility of compound X suggested that it might be a monohydroxy fatty acid, selected standards were subjected to straight phase h.p.l.c. and their retention times compared with that of compound X and the 12-HETE standard (Table 1). On this basis, twelve standards with straight phase h.p.l.c. retention times similar to that of compound X were retested in the described reversed phase system. Five of these standards showed reversed phase h.p.l.c. retention times near to that of 12-HETE, which co-eluted with compound X in this system: 9-hydroxyoctadecadienoic acid (9-HODD), 8-hydroxyeicosatetraenoic acid (8-HETE), 9-HETE, 11-hydroxy-12,14,17-eicosatrienoic acid and 8-hydroxy-9,11,14-eicosatrienoic acid (Table 2).

The four derivatised samples obtained after successive straight and reversed phase h.p.l.c. purification of scale extracts were subjected to g.c.m.s., with selective monitoring of ions characteristic of the MeTMS derivatives of the five monohydroxy fatty

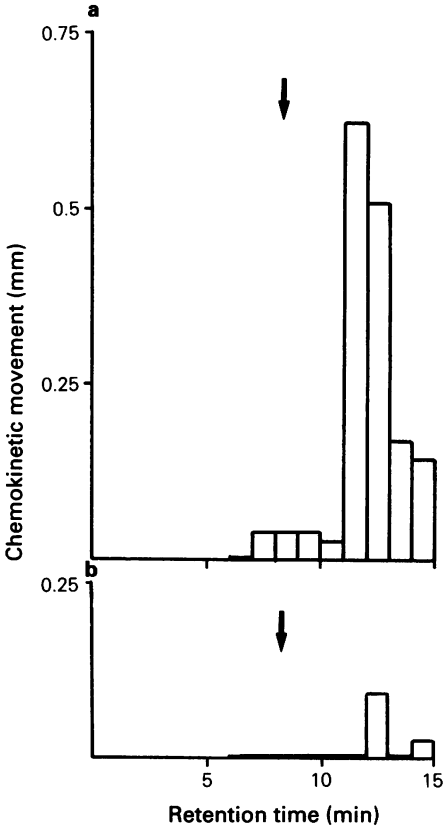


**Figure 3** Chemokinetic activity in straight phase h.p.l.c. fractions after purification of extracts of chamber fluid from the lesional (a) and clinically uninvolved (b) skin of a volunteer with psoriasis. Collection of the first h.p.l.c. fraction began 6 min after injection in each case. Each fraction was evaporated, redissolved in 0.3 ml HEPES-buffered MEM and assayed for chemokinetic activity without further dilution. Activity has been expressed as the distance moved by leucocytes less random movement in the presence of HEPES-buffered MEM alone. The mean of duplicate observations is given for each h.p.l.c. fraction. The arrows show the elution times of standard 12-hydroxyeicosatetraenoic acid (12-HETE).

**Table 1** Retention times of monohydro(pero)xy fatty acids on straight phase h.p.l.c.

| *Parent fatty acid* | | *Position of hydro(pero)xy group* | *Retention time* (min) |
|---|---|---|---|
| Arachidonic acid | (C20 : 4 ω-6) | 12 | 8.8 |
| Eicosadienoic acid | (C20 : 2 ω-6) | 15 | 10.4 |
| Arachidonic acid | (C20 : 4 ω-6) | 12-OOH* | 10.6 |
| Linolenic acid | (C18 : 3 ω-3) | 13 | 10.8 |
| Dihomo-γ-linolenic acid | (C20 : 3 ω-6) | 11 | 10.9 |
| Linoleic acid | (C18 : 2 ω-6) | 13 | 11.2 |
| Eicosapentaenoic acid | (C20 : 5 ω-6) | 11 | 11.3 |
| Eicosadienoic acid | (C20 : 2 ω-6) | 11 | 11.4 |
| Linolenic acid | (C18 : 3 ω-3) | 16 | 11.5 |
| Eicosatrienoic acid | (C20 : 3 ω-3) | 11 | 12.0† |
| γ-Linolenic acid | (C18 : 3 ω-6) | 10 | 12.7† |
| Linolenic acid | (C18 : 3 ω-3) | 12 | 12.9† |
| Eicosatrienoic acid | (C20 : 3 ω-3) | 18 | 12.9† |
| Eicosapentaenoic acid | (C20 : 5 ω-3) | 9 | 13.3† |
| γ-Linolenic acid | (C18 : 3 ω-6) | 9 | 13.4† |
| Arachidonic acid | (C20 : 4 ω-6) | 9 | 13.7† |
| Linolenic acid | (C18 : 3 ω-3) | 9 | 14.0† |
| Dihomo-γ-linolenic acid | (C20 : 3 ω-6) | 8 | 14.5† |
| Eicosapentaenoic acid | (C20 : 5 ω-3) | 8 | 14.6† |
| Linoleic acid | (C18 : 2 ω-6) | 9 | 14.9† |
| Arachidonic acid | (C20 : 4 ω-6) | 8 | 15.5† |

* 12-Hydroperoxyeicosatetraenoic acid (12-HPETE).
† The fractions containing compound X eluted 3–6 min after the retention time of 12-hydroxyeicosatetraenoic acid (12-HETE).

acid standards which co-eluted with compound X in both h.p.l.c. systems (Table 3). Ion chromatograms indicative of the presence of the derivatives of 9-HETE and 9-HODD were consistently obtained (Figure 5), but none of the other three monohydroxy fatty acid derivatives was detectable.

The chemokinetic activity in response to 9-HODD, 9-HETE and 12-HETE (racemic forms) is shown in Figure 6. 9-HODD and 9-HETE showed low activity in this assay compared on a molar basis with 12-HETE.

## Discussion

Extracts of scale and chamber fluid from lesional psoriatic skin have now been shown to contain biologically active amounts of a previously undescribed monohydroxy fatty acid-like substance which is capable of stimulating neutrophil movement in a dose-related manner, and which is referred to as compound X (Figures 1–4). Although psoriatic scale is essentially metabolically inert tissue, it appears unlikely that compound X is an artefact produced in

**Table 2** Retention times of monohydroxy fatty acids on reversed phase h.p.l.c.

| *Parent fatty acid* | | *Position of hydroxy group* | *Retention time* (min) |
|---|---|---|---|
| Linolenic acid | (C18 : 3 ω-3) | 9 | 8.2 |
| Linolenic acid | (C18 : 3 ω-3) | 12 | 8.5 |
| γ-Linolenic acid | (C18 : 3 ω-6) | 9 | 8.5 |
| γ-Linolenic acid | (C18 : 3 ω-6) | 10 | 8.8 |
| Eicosapentaenoic acid | (C20 : 5 ω-3) | 8 | 8.9 |
| Eicosapentaenoic acid | (C20 : 5 ω-3) | 9 | 9.4 |
| Linoleic acid | (C18 : 2 ω-6) | 9 | 10.5* |
| Arachidonic acid | (C20 : 4 ω-6) | 8 | 11.7* |
| Arachidonic acid | (C20 : 4 ω-6) | 9 | 12.4* |
| Eicosatrienoic acid | (C20 : 3 ω-3) | 11 | 12.9* |
| Dihomo-γ-linolenic acid | (C20 : 3 ω-6) | 8 | 13.5* |
| Eicosatrienoic acid | (C20 : 3 ω-3) | 18 | 14.3 |

* The retention time of compound X was between 11–13 min in the reversed phase h.p.l.c. system used.

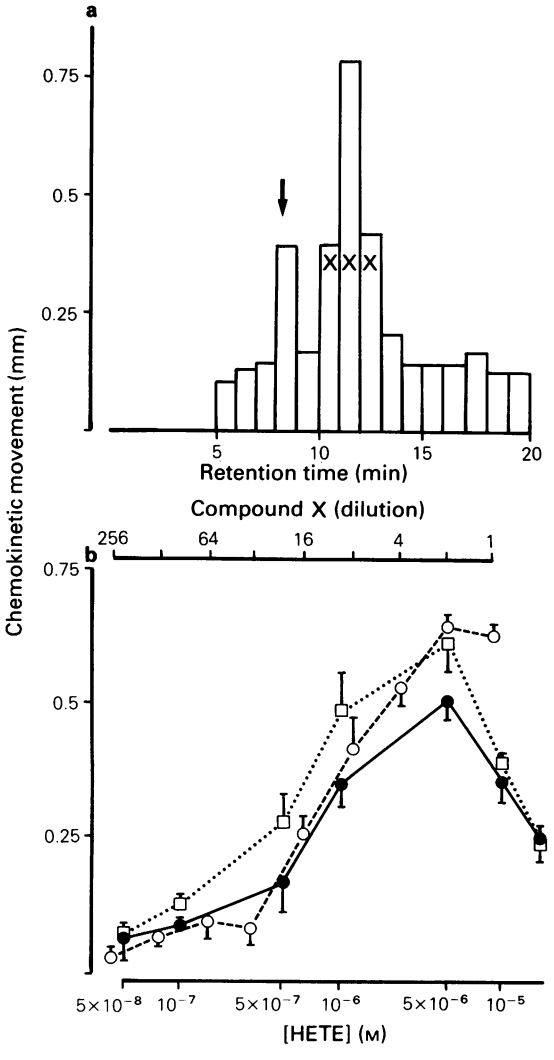


**Figure 4** Chemokinetic activity in an extract of psoriatic scale (500 mg) after purification by straight phase h.p.l.c. Collection of the first h.p.l.c. fraction began 5 min after injection. One quarter of each fraction was evaporated, redissolved in 0.3 ml HEPES-buffered MEM and assayed for chemokinetic activity after five fold dilution (a). Chemokinetic activity has been expressed after subtraction of background values obtained in the presence of HEPES-buffered MEM alone. Each point represents the mean of duplicate observations. The elution time of standard 12-hydroxyeicosatetraenoic acid (12-HETE) is shown by the arrow. The remaining three-quarters of the three fractions containing compound X (X) were pooled and assayed for chemokinetic activity in doubling dilution as described (○) and the activities of racemic 12-HETE (●) and 12(**R**)-HETE (□) were determined in the same assay (b). Each point shows the mean of triplicate estimations after subtraction of background activity. The vertical lines represent s.d.

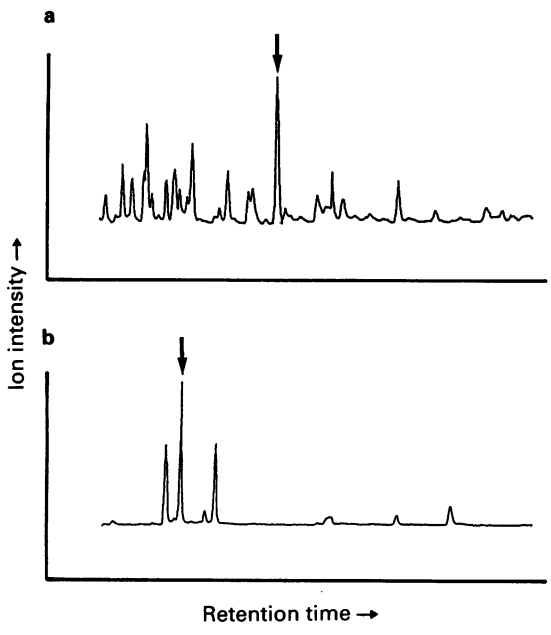


**Figure 5** Representative chromatograms obtained when h.p.l.c. fractions containing compound X were analysed by g.c.m.s. with monitoring of ions characteristic of the methyl ester, trimethylsilyl ether (MeTMS) derivatives of 9-hydroxyeicosatetraenoic acid (9-HETE; m/z 255, a) and 9-hydroxyoctadecadienoic acid (9-HODD, m/z 292, b). The arrows show the retention times of the MeTMS derivatives of standard 9-HETE (a) and 9-HODD (b) under the same g.c.m.s. conditions.

such tissue, in view of the finding that it is released into skin chambers placed over abraded, washed lesions (Figure 3). As previously discussed (Brain *et al.*, 1984b), the release of biologically active material into the described skin chambers suggests that such material is formed in the deeper, viable epidermal layers, and not simply in the surface scale. Furthermore, recent work has shown that human epidermal cell suspensions incubated *in vitro* are capable of producing material similar to compound X (R.D.R. Camp *et al.*, unpublished observations).

The maximal chemokinetic effect of compound X is similar to that of 12(**R**)-HETE (Figure 4) which is the major 12-HETE stereoisomer present in psoriatic scale (Woollard, 1986) and which possesses greater chemokinetic potency than 12(**S**)-HETE (Cunningham & Woollard, 1987). Compound X was found in all samples analysed except for chamber fluid from clinically normal skin of volunteers with psoriasis (Figure 3). It is chromatographically distinct from the chemoattractant eicosanoids leukotriene $B_4$ and 12-HETE, both of which have been identified previously in extracts of psoriatic lesions and, in view of its ability to stimulate leucocyte

**Table 3** Fragment ions characteristic of the methyl ester, trimethylsilyl ether derivatives of the five monohydroxy fatty acids monitored by g.c.m.s.

| *Monohydroxy fatty acid* | *Ion monitored* (m/z) | *Structure of ion* |
|---|---|---|
| 9-HODD | 292 | $[M\text{-}(CH_3)_3SiOH]^+$ |
| 8-HETE | 265 | $[M\text{-}CH_2CHCH(CH_3)_3COOCH_3]^+$ |
| 9-HETE | 255 | $[M\text{-}CH_2CHCHCH_2CHCH(CH_3)_4CH_3]^+$ |
| 11-Hydroxy-12,14,17-eicosatrienoic acid | 223 | $[M\text{-}CH_2(CH_3)_8COOCH_3]^+$ |
| 8-Hydroxy-9,11,14-eicosatrienoic acid | 265 | $[M\text{-}CH_2CHCH(CH_3)_3COOCH_3]^+$ |

9-HODD = 9-hydroxyoctadecadienoic acid and 8-HETE = 8-hydroxyeicosatetraenoic acid.

movement, may contribute to the inflammatory changes seen in psoriasis. It is noteworthy that compound X cannot be resolved from 12-HETE on reversed phase h.p.l.c., as illustrated by the profile of biological activity obtained after prior removal of 12-HETE from scale extracts by straight phase h.p.l.c. (Figure 2).

The retention times of selected monohydroxy fatty acid standards were determined in the two h.p.l.c. systems used. 12-HPETE, which is the precursor of 12-HETE in lipoxygenase reactions, was also selected in view of the findings of large amounts of the latter material in psoriatic scale (Hammarström *et al.*, 1975; Camp *et al.*, 1983; Barr *et al.*, 1984). Of the standards tested, five co-eluted with compound X in both h.p.l.c. systems. Selected ion monitoring g.c.m.s. analysis was carried out on MeTMS derivatives of residues containing biologically active amounts of compound X, obtained after successive purification of psoriatic scale extracts by straight and reversed phase h.p.l.c., and the derivatives of 9-HETE and 9-HODD were detected (Figure 5). Relative to 12-HETE, 9-HETE showed low activity in the agarose microdroplet assay (Figure 6). Previous quantitative g.c.m.s. analysis of lesional psoriatic scale extracts has shown that there is approximately ten times more 12-HETE than 9-HETE present ($1589 \pm 617$ and $111 \pm 31$ ng $100\,mg^{-1}$ scale, respectively; mean $\pm$ s.e.mean, $n = 7$; Cunningham *et al.*, 1985). The chemokinetic activity due to compound X, which was consistently greater than that due to 12-HETE in the samples assayed, cannot therefore be explained by the presence of 9-HETE, or of 9-HODD, which produced only minimal activity in the chemokinesis assay (Figure 6).

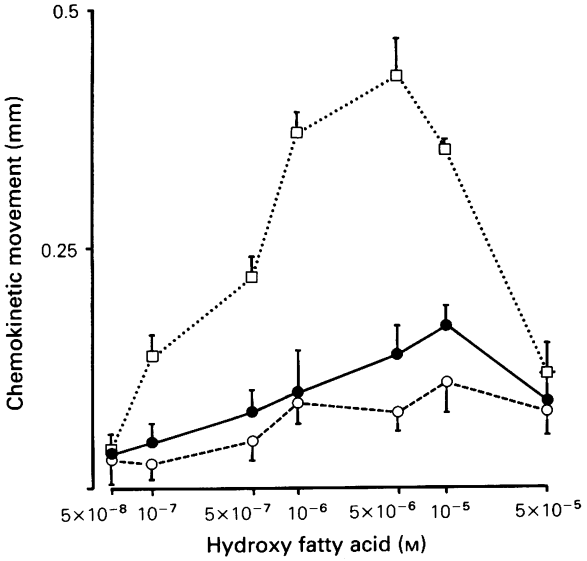


**Figure 6** Chemokinetic activity in response to standard preparations of racemic 9-hydroxyoctadecadienoic acid (○), 9-hydroxyeicosatetraenoic acid (9-HETE, ●) and 12-HETE (□). Activity has been expressed as the distance moved by leucocytes less random movement in the presence of HEPES-buffered MEM alone. Each point represents the mean of triplicate observations, and vertical lines indicate s.d. Similar results were obtained in a second experiment.

The identity of compound X, its mechanism of synthesis and its specificity to psoriasis, remain to be established. The finding of another compound which stimulates neutrophil movement in psoriatic lesions, in addition to those previously described, highlights the complexity of inflammatory mediator production in this disease, and further suggests that drugs with a broad spectrum of anti-inflammatory action may be more effective therapeutically in psoriasis than compounds which inhibit the formation or effects of a single compound.

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