Studies were performed to investigate the prohormone/pro-
protein convertase (PC)-inhibitory properties of chemical con-
stituents of the medicinally active plant Andrographis paniculata
(AP; from the family Acanthaceae), also known as ‘King of
Bitters’. Among the individual components tested against the
clinically important convertases, furin and PC1, neandro-
grapholide (a C1 O-glucoside derivative of the major constituent
andrographolide) exhibited the highest inhibitory action with an
IC50 of 53.5 µM against furin. The data further revealed that
although andrographolide, the major bitter principle of AP,
exhibited a relatively small enzyme inhibition (IC50 = 1.0 mM
and KI = 200 µM against furin), upon succinylation, its in-
hibitory action against the above convertases was enhanced
significantly with a KI in the low micromolar range (< 30 µM),
suggesting that a specific structural modification of the andro-
grapholide skeleton may be exploited to develop a new class of
non-peptide inhibitors of PCs. When tested against PC7, these
succinoylated derivatives of andrographolide also displayed
strong inhibitory action, with KI values again in the low
micromolar range. This potentially interesting observation may
be attributed to the reported anti-HIV property of 14-dehydro-
andrographolide succinic acid monoester (DASM). It is suggested
here that DASM, by virtue of this protease inhibitory property,
possibly acts by suppressing the proteolytic cleavage of envelope
glycoprotein gp160 of HIV, which is known to be PC-mediated,
particularly by furin and PC7.

Key words: antiviral property, inhibition constant, labdane
diterpines, protease inhibitor.

INTRODUCTION

Intracellular endoproteolytic processing at specific sites (usually
C-terminal of a pair of basic amino acids, such as Lys-Arg or
Arg-Arg) is a common post-translational modification of mem-
brane and secretory proteins, which are synthesized initially as
inactive propolypeptides or proproteins. These proteins include
a wide range of hormones, neuropeptides, growth factors,
coagulation factors, cell-surface receptor proteins and adhesion
molecules, as well as the surface viral glycoproteins [1]. The long
quest for the processing enzyme(s) of these proproteins culmi-
nated recently in the discovery and molecular characterization of
a family of bacterial subtilisin/yeast kexin-like enzymes, col-
lectively known as proprotein/ prohormone convertases (PCs; reviewed in [1–3]). To date, seven members of this family have been
identified, namely PC1/PC3, PC2, furin, paired-amino-
acid-cleaving enzyme (PACE)4, PC4, PC5/PC6 and PC7. On the
basis of the distribution among tissues, co-localization and
studies in vitro and in vivo, a number of these convertases are
implicated in many disease states and vital functions [4–6]; for
example, PC5 is involved in atherosclerosis [6], PC1 in obesity
[6–8], furin in viral infection [6] and embryogenesis [9], PC4 in
fertilization [10], PACE4 and PC7 in breast cancer [4,6,11], and
PC7, PC5 and furin in Alzheimer’s disease, hypertension and
neoplasia [6]. These findings suggest that PCs are important
therapeutic targets. Many studies in vitro have already indicated
that furin-specific inhibitors, such as az-PDX (a double arginine
mutant of az-antitrypsin Pittsburgh [12,13] at positions 355 and
358), are able to block significantly the processing of viral
glycoprotein gp160 of HIV mediated by furin [14–16] and,
consequently, its membrane fusogenic properties [12].

In the light of these studies, we became interested in the
development of specific inhibitors of PCs. Our efforts had already
yielded several peptide-based inhibitors [17–22] for PC1 and
furin. During the development of these peptide inhibitors, we
realized their limitations, owing to their proteolytic instability
and poor membrane permeance. We then started looking for
non-peptide inhibitors from natural sources, particularly from
the medicinal plants, which in the past had provided a large
number of therapeutic agents. One plant, Andrographis paniculata
(AP, from the family Acanthaceae; also known as ‘King of
Bitters’ or ‘Rice Bitters’) [23], has especially attracted our
attention because of its widespread use in south-east Asian and
east Indian countries in a variety of human illnesses. These range
from acute hepatitis, bacillary dysentery, digestive ailments with
infectious conditions, such as bacterial diarrhoea, meningitis,
choriocarcinoma (a form of cancer), dyspepsia, influenza, malaria, filaria, and many other acute inflammatory conditions [24–34]. Previous studies have indicated the presence of andrographolide (the major bitter principle) [35], a diterpene of the labdane family, along with its 19-O-glucoside derivative, called andrographside [35]. A number of related diterpines and some flavonoids were also isolated from AP as minor constituents [36–42]. In 1988, Chang and Yeung [43] and, later, Yao et al. [44] reported in vitro anti-viral properties of a crude extract of AP towards HIV. It was further revealed that dehydroandrographolide succinic acid monoester (DASM), prepared from andrographolide, exhibited vastly improved anti-HIV properties [45], at concentrations ranging from 50–200 μg/ml. DASM interfered with the development of HIV by a mechanism other than induction with interferon. It was noted that it did not inactivate cellular HIV or inhibit reverse transcriptase activity, but it partly interfered with both the HIV-induced cell fusion and binding of HIV to the T-helper-cell line (H9), and in phytohaemagglutinin-activated blood mononuclear cells from HIV-seronegative persons. Furthermore, it also partly inhibited HIV replication after the virus had become attached to cell receptors [45]. Overall, DASM exerts its suppressive effect on HIV growth by interference with both the binding of virions to cells and a step in the viral replication cycle subsequent to virus–cell binding. However, its effect on the processing of viral glycoprotein gp160 was never investigated. We envisage that DASM might interfere in this processing, which is known to be mediated by PCs such as furin and/or PC7 [12,15,46]. This prompted us to examine the possibility of inherent PC-inhibitory properties in DASM and andrographolide-related substances from AP. In the present study, we present data which demonstrate that andrographolide-related labdane diterpines possess PC1-, PC7- and furin-inhibitory properties that are enhanced significantly upon the selective formation of derivatives, such as succinoylated ones.

**MATERIALS AND METHODS**

**Materials**

A number of chemical constituents of AP used in the present study, namely andrographolide, 14-deoxy-11,12-didehydroandrographolide, neoandrographolide and 14-deoxyandrographolide, were generously given by Professor J. D. Connolly (Department of Chemistry, University of Glasgow, Scotland, U.K.). Andrographolide was also purchased from Aldrich Chemical Company (Milwaukee, WI, U.S.A), whereas andrographolide-triacetate, phglantholide (2α-hydroxy-3-deoxy-andrographolide-19-galactoside), its triacetate and 2-O-galactoside derivatives were gifts from Professor A. K. Barua (Bose Institute, Calcutta, India).

The fluorogenic substrate 1-pyroglyutamyl-l-arginyl-l-threonyl-l-lysyl-l-arginyl-4-methyl-7-coumaranamide (pGlu-Arg-Thr-Lys-Arg-MCA) was purchased from Peptides International (Louisville, KY, U.S.A.). Reverse-phase (RP)-HPLC was performed using a CSC-Exsil analytical C18 column (25 cm × 0.46 cm; Chromatography Specialty Corp., St-Laurent, PQ, Canada). The buffer system consisted of an acq. 0.1% (v/v) trifluoroacetic acid (TFA) solution and an organic phase of CH3CN, also containing 0.1% (v/v) TFA. The elution was performed by using a linear gradient from 5–60% organic phase in 60 min, following a 5-min isocratic-gradient step at 5% organic phase; the flow was adjusted to 1 ml/min. The separation was monitored by measuring UV absorbance at 225 nm. 1H-NMR spectra (in deuterated DMSO-D4) and mass spectra were recorded on Varian Associate Bruker 400 MHz and MS-50 HMTCTA (chemical-ionization mode) instruments respectively.

**Sources of convertases**

The endoproteases used in the present study, human (h)PC1 and furin, were obtained from the medium of the GH4C1 cell line, following infection with respective recombinant vaccinia virus [17,18], whereas recombinant hPC7 was obtained by following a procedure reported previously [47]. Enzymically active mouse (m)PC1 was recovered from the medium of baculovirus-infected Sf9 insect cell lines (A. Boudreauil, N. G. Seidah, M. Chretien and C. Lazure, unpublished work). The enzymic activity of PC1, PC7 and furin was measured by fluorimetric assay, as described previously [18,47].

**Preparation of succinoyl ester of andrographolide (SEA)**

The conditions used were essentially the same as those described previously for the preparation of DASM [45]. Briefly, andrographolide (500 mg; 1.43 mmol) was dissolved in pyridine (5 ml). Succinic anhydride (450 mg; 4.5 mmol) was added and the mixture was stirred at ambient temperature for 20 h. Solvent was removed under pump and the residue obtained was washed extensively with ether. The ether-insoluble material was mixed twice with water (50 ml fractions) and filtered. The filtrate was then freeze-dried to yield crude SEA as a powder (568 mg; 70% yield).

**Purification of crude SEA**

Crude SEA (20 mg) was dissolved in DMSO (1 ml) and then diluted with water to a final concentration DMSO of 10%. RP-HPLC was performed under the conditions described above, except that a flow rate of 2 ml/min with a semi-preparative C18 column (25 cm × 1.0 cm; Chromatography Specialty Corp.) was used. Peaks (five in all, including the unreacted andrographolide) were collected, freeze-dried and characterized by both MS and 1H-NMR spectral analyses.

**Analysis of fractions**

Note that the absolute configurations given below at the C-14 position in SEA-1 and SEA-2 could not be fully established and are therefore tentatively assigned as 14z and 14f/ for fractions 1 and 2 respectively. The value for the retention time (Rt) representative of the C18 semi-preparative column is given within parentheses, whereas the other value refers to time on a C18 analytical column. The peak intensities of the signals (relative to each other) in mass spectral data are characterized as follows: vw, very weak; w, weak; ms, medium; s, strong; vs, very strong.

**Fraction-1 (SEA-1)**

For andrographolide 3α,14z,19-O-trisuccinate, Rt = 40.16 (33.9) min. MS analysis: m/z 651 (vw); 633 (s); M + H+ 651 (ms); M + H2O+ 615 (ms); M + 2H2O+ 591 (ms); M + H - COOCH3CH2COOH+ 445 (ms). For 1H-NMR analysis, (i) CH3 groups: δ 0.76 (3H), 1.18 (3H), 1.8 (3H); (ii) ring CH3/CH2 groups: δ 1.20–1.75 (7H); (iii) allylic H atoms: δ 1.98 (1H), 2.01 (1H), 2.46 (1H), 2.52 (1H) and 2.59 (1H); (iv) lactonyl CH2 groups: 3.96 (1H) and 3.36 (1H); (v) carbonyl H atoms: δ 4.14 (1H), 4.51 (2H); (vi) succinyl CH2 groups: δ 1.80–2.35 (12H); (vii) olefinic H atoms: δ 4.70 (1H), 4.90 (1H) and 6.74 (1H), 4.9 (1H) and 4.7 (1H).

**Fraction-2 (SEA-2)**

For andrographolide 3α,14f,19-O-trisuccinate, Rt = 41.59 (34.95) min. MS analysis: m/z 651 (vw); M + H+ 633 (vs;
$M + H – H_2O^+$, 615 (ms; $M + H – 2H_2O^+$), 519 (w, ms; $M + H – CH_OCOCH(CH_2COOH)$). 1H-NMR data are inconsistent with this structure.

Fraction-3 (SEA-3)

For unreacted andrographolide, $R_t = 42.06$ (36.0) min. MS analysis: $m/z$ 351 ($M + H^+$). 1H-NMR data are in agreement with its structure.

Fraction-4 (SEA-4)

For monopyridinium salt of andrographolide 3z,14z$/beta$,19-O-trisuccinate, $R_t = 42.8$ (36.6) min, MS analysis: $m/z$ 731 (vw; $M + H^+$), 637 (vs; $M + H – Py – CH_2$). 622 ($M + H – Py – 2CH_2$). 1H-NMR data are in agreement with its structure.

Fraction-5 (SEA-5)

For DASM, $R_t = 50.99$ (44.0) min. MS analysis: $m/z$ 533 (vs, $M + H^+$). 1H-NMR data are inconsistent with its structure.

**Enzyme-inhibition studies**

Determination of kinetic parameters, $K_i$ and IC$_{50}$ values

The kinetic parameters $K_i$ and IC$_{50}$ were determined according to the procedure described previously [17,18]. For determination of IC$_{50}$ values, inhibitor concentrations were varied over a range wide enough to generate a residual protease activity of 20–80% of the original. $K_i$ values were derived from Dixon and Cornish-Bowden plots, using the fluorogenic substrate pGlu-Arg-Thr-Lys-Arg-MCA at a final concentration of 15 $\mu$M and 45 $\mu$M concentrations, or calculated from the IC$_{50}$ values by using the equation $K_i = IC_{50}/(1 + [S]/K_m)$, applicable for reversible competitive inhibition [24], where $K_m$ is the Michaelis–Menten constant.

**RP-HPLC of some AP constituents and their derivatives**

For RP-HPLC analysis of all materials, including several components of AP, such as andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide, phlogantholide, phlogantholide triacetate and phlogantholide glucoside, the samples were dissolved in DMSO and then diluted with an appropriate volume of water to give a final concentration of 1.0 $\mu$g/$\mu$l in 10% (v/v) DMSO. HPLC was performed on 20$\mu$l of each stock solution, using the conditions as described above.

**RESULTS**

Effect of individual components of AP towards furin, PC1 and PC7 activity

A number of individual components of AP, including andrographolide (the major constituent), were examined for any potential inhibitory response towards the proteolytic activities of clinically important PCs, i.e. furin, PC1 and, in some cases, PC7. Some of these compounds, which share a common diterpene labdane skeleton [35,36], showed a weak-to-moderate inhibition of these convertases when measured against pGlu-Arg-Thr-Lys-MCA substrate, with IC$_{50}$ values ranging from the low micromolar to the millimolar level (Table 1). Among the compounds tested, neoandrographolide was found to be the most potent furin inhibitor, with a measured IC$_{50}$ value of 53.5 $\mu$M. This is nearly 20-fold more potent than andrographolide (IC$_{50}$ = 1.0 mM and $K_i = 200$ $\mu$M against furin). However, andrographolide inhibits hPC1 (IC$_{50} = 750$ $\mu$M) more strongly than furin (Table 1). To date, to our knowledge it represents the first non-peptide inhibitor of PCs, and could be considered as an important precursor structure that might require further structural modification for enhancement of its enzyme-inactivation

<table>
<thead>
<tr>
<th>List of compounds</th>
<th>Furin</th>
<th>PC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrographolide</td>
<td>1.0 $\mu$M</td>
<td>750 $\mu$M</td>
</tr>
<tr>
<td>Andrographolide triacetate</td>
<td>&gt; 2.0 $\mu$M</td>
<td>a</td>
</tr>
<tr>
<td>Neoandrographolide (14-deoxyandrographolide)</td>
<td>53.5 $\mu$M</td>
<td>—</td>
</tr>
<tr>
<td>Dehydroandrographolide</td>
<td>&gt; 4.0 $\mu$M</td>
<td>—</td>
</tr>
<tr>
<td>14-Deoxyandrographolide</td>
<td>634 $\mu$M</td>
<td>205 $\mu$M</td>
</tr>
<tr>
<td>Phlogantholide</td>
<td>398 $\mu$M</td>
<td>b</td>
</tr>
<tr>
<td>Phlogantholide glucoside acetate</td>
<td>521 $\mu$M</td>
<td>c</td>
</tr>
</tbody>
</table>

Table 1 Comparison of the ability of various labdane diterpines isolated from AP to inactivate PC1 and furin

IC$_{50}$ values, shown here as averages, were determined either in duplicate or in triplicate according to the procedure described in the Materials and methods section using pGlu-Arg-Thr-Lys-Arg-MCA at a final concentration of 100 $\mu$M, a, b and c denote 11%, 10% and 18% inhibition respectively at 400 $\mu$M concentration of the inhibitor.
property. It should be emphasized that, unlike most other constituents of AP, neoandrographolide possesses a unique C₁₆-O-glucosyl moiety, that perhaps could explain its increased level of furin inactivation. This observation indicates that the specific derivative formation of the C₁₆-OH group, such as glycosylation in this case, might lead to a molecule with a greater capacity to inhibit furin. In fact, as demonstrated below, succinoylation of this group also improves its PC1- and furin-inhibitory properties to a significant extent. In comparison, PC7 is inhibited relatively weakly by the components of AP, including neoandrographolide (IC₅₀ ranging from 500 μM to 2 mM; results not shown).

Succinoylation of andrographolide

In 1991, Chang et al. [45] reported that, upon treatment with succinic anhydride, andrographolide yielded DASM, which showed potent anti-viral properties against HIV-1 and HIV-2. It was assumed that DASM was a mixture of two isomeric esters (structures IIa and IIb), as shown in Figure 1. However, no attempts were made to purify and characterize the individual components of DASM. When we repeated the reaction (see the Materials and methods section), we obtained a crude material (structures; see also the Materials and methods section), we obtained a crude material called SEA. Its mass spectrum indicated the presence of a mixture of mainly trisuccinyl ester of andrographolide (Mₘ = 651), in addition to small amounts of monosuccinate (Mₘ = 451), disuccinate (Mₘ = 551) and 14-dehydrodisuccinate (Mₘ = 533).

RP-HPLC of crude SEA (results not shown) yielded four major fractions (SEA-1, SEA-2, SEA-4 and SEA-5), in addition to the unreacted andrographolide (SEA-3), as confirmed by HPLC and MS with an authentic sample. The four new fractions, SEA-1, SEA-2, SEA-4 and SEA-5, were identified by MS and H-NMR spectroscopic analyses as being respectively: andrographolide-3,14,19-trisuccinate [III, Rₜ = 40.16 min, m/z 651 (M + H)]; andrographolide-3,14β,19-trisuccinate [IV, Rₜ = 41.59 min, m/z 651 (M + H)], monopyridinium salt of andrographolide-3,14β,19-trisuccinate [shown as V, out of several possible structures; Rₜ = 42.8 min, m/z 731 (M + H)⁺] and 14-dehydroandrographolide 3,19-disuccinate [VI, Rₜ = 50.99 min, m/z 533 (M + H)⁺]; where the configurations at the C-14 position of structures III and IV (α and β respectively) are only tentatively assigned. Therefore, in the present study, the major product of succinoylation of andrographolide was 3,14,19-tri-O-succinate, whereas the β-elimination of the 14-hydroxy group occurred only as a minor side reaction, in contrast with that reported by Chang et al. [45], in which a complete elimination of this hydroxy group of DASM was observed.

Inhibition of PC1, PC7 and furin by crude SEA

The amidolytic activity of h/mPC1, hPC7 and hfurin, as measured with the fluorogenic substrate, pGlu-Arg-Thr-Lys-Arg-MCA, were all inhibited relatively strongly by crude SEA in a concentration-dependent manner, as shown in Figure 2 for hPC1 using a stop-time assay and in Figure 3 for mPC1, using an initial-rate assay. SEA inhibited PC1 (IC₅₀ = 150 μM for mPC1 and 50 μM for hPC1) much more strongly than andrographolide itself (IC₅₀ = 750 μM with hPC1) under identical conditions. This observation reinforced the notion that the formation of hydroxy group derivatives of andrographolide enhances its inhibitory potency towards PCs. As shown in Figure 3, purified baculovirus-derived mPC1 was gradually inhibited by treatment with increasing amounts of crude SEA. The observed 3-fold difference in the degree of inhibition of hPC1 and mPC1 by SEA might be explained by the fact that there are varying levels of 85 and 66 kDa forms of PC1 in the two enzyme preparations, which were derived from two different cell lines. It should be pointed out that the 66 kDa form is enzymically more active than the 85 kDa form [48], and therefore the IC₅₀ value is dependent upon the relative amounts of the two forms present.

© 1999 Biochemical Society
Further studies by us have indicated that crude SEA is also capable of inhibiting PC7, another clinically important convertase. In fact, we found that crude SEA inhibited hPC7 with a measured $K_i$ of 38 µM compared with 50 µM and 42 µM for hPC1 and hfurin respectively (Table 2). Thus it seems that, as an inhibitor, SEA is not very selective towards any particular member of the PC family.

$K_i$ values of various purified fractions of SEA against convertases

All four succinoyl derivatives of andrographolide (SEA-1, -2, -4 and -5) were capable of inhibiting PC1, PC7 and furin activity, as measured with pGlu-Arg-Thr-Lys-Arg-MCA as the substrate. This is clearly demonstrated by the Dixon plots in Figure 4, showing the inactivation of hPC1 by various purified SEA fractions using two different substrate concentrations, namely 15 µM (S1) and 45 µM (S2). $K_i$ values for these derivatives were calculated to range from 10 to 24 µM. Among them, SEA-2 (IV, andrographolide 3,14β,19-trisuccinate), possessed the greatest inhibitory action, with a $K_i$ of 10.1 µM against hPC1. The Dixon plots also confirmed the reversible competitive nature of this inhibition. As shown in Table 2, the individual fractions of SEA displayed a much greater selectivity for inhibition of furin, as compared with PC1 and PC7. In particular, SEA-4 (V; as a representative structure, monopyridinium salt of andrographolide 3x,14β,19-O-trisuccinate), demonstrated a high degree of potency towards furin inhibition, with a $K_i$ = 2.6 µM. In fact, SEA-4 is a 9–10-fold more potent inhibitor of hfurin than either hPC1 or hPC7. Seemingly, the presence of a positively charged pyridine ring in one of the three succinic acid functions (3a, 14β or 19) of SEA-4 contributes significantly towards the enhancement of the furin-inhibitory property. It should be mentioned that, at this stage, the precise location of this pyridinium ring in SEA-4 is not known. With the exception of SEA-4, all other fractions of SEA, as well as crude SEA material, display hardly any significant specificity, in terms of enzyme inhibition towards any of the three convertases tested to date.

**DISCUSSION**

Our studies revealed that, among the various compounds of AP tested, neoandrographolide, a C$_3$-diterpine glucoside, possesses the highest inhibitory action against PCs, particularly furin. This is the first report of inactivation of PCs by diterpines although, previously, a limited number of polycyclic triterpenes (C$_{30}$ atoms), such as ursolic acid, was shown to inhibit the proteolytic activity of human leucocyte elastase [49]. Our data further revealed that although andrographolide itself, the major constituent of AP, exhibited only a relatively weak inhibitory action towards PC1 and furin, this property was significantly enhanced upon the formation of the hydroxy group derivatives via succinoylation. This observation provided the first scientific rationale for the reported anti-viral properties of crude extract of AP and, in particular, DASM, a crude mixture of succinoylated derivatives of dehydroandrographolide, towards HIV-1 and HIV-2 [43–45]. As explained previously [12,14], viral infection requires interaction of viral-coat glycoproteins, such as gp160 in the case of HIV, with proteins at the plasma membrane of susceptible cells. Some of these glycoproteins need to be processed proteolytically in order to be able to mediate viral infectivity. There is now
sufficient experimental evidence to suggest that this processing is mediated by cellular proteases at sites (Arg-Glu-Lys-Arg) for gp160, compatible with the cleavage specificity of furin [9,12] and PC7 [47]. This post-translational cleavage is one of several events that determine the viral infectivity and virulence. Previously, it was demonstrated by studies both in vivo and in vitro that furin- and PC7-directed inhibitors were able to block significantly the proteolytic processing of gp160 [12–14], and therefore these displayed anti-viral properties towards HIV. Likewise, it may be argued that the SEAs, succinylated derivatives of andrographolide, which display powerful inhibitory properties towards PCs, particularly furin, are equally capable of preventing the furin-mediated processing of gp160 and therefore display anti-HIV properties. In fact, preliminary studies in vitro performed in our laboratory seem to suggest that administration of crude SEA is not only capable of partially preventing the formation of gp120 and gp41 from gp160 (E. Decroly, A. Basak and N. G. Seidah, unpublished work), but might also have effects on the processing of other protein precursors.

Our observation that formation of the free hydroxy group derivatives of andrographolide significantly enhanced its ability to inhibit convertase activity (compare SEAs versus andrographolide) has now provided an opportunity for the development of new lead compounds as PC inhibitors. Therefore natural products with or without chemical modification might offer a great potential for the future development of new lead compounds as PC inhibitors.

**Conclusion**

In conclusion, this study has revealed that diterpines of the labdane family could represent an interesting class of non-peptide inhibitors of PCs, namely PC1, PC7 and furin. To our knowledge, this is the first report of protease-inhibitory properties of diterpines (C29 compounds). Though relatively modest in their action, it might be possible to enhance their inhibitory properties by further structural modification. This study has now provided a scientific rationale for the reported anti-viral properties of AP compounds, and particularly some of the synthetic derivatives. Therefore natural products with or without chemical modification might offer a great potential for the future development of new lead compounds as PC inhibitors.

We thank Dr. M. Evans, Chemistry Department, Montréal University, Canada, and Dr. K. Carpenter, Clinical Research Institute of Montréal (IRCM), Canada, for recording the mass and NMR spectra respectively. Thanks are also due to D. Gauthier, D. Savaria and A. Lemieux, of the Clinical Research Institute for technical assistance. We also thank Dr. Jon Scott Munger and Mei Zhong for the recombinant PC7. We acknowledge the support from Dr. C. Lazare, Director of the Laboratory of Structure and Metabolism of Neuropeptides, Clinical Research Institute. Finally, we thank the following persons for their interest in the work: Dr. J. Archambault, Université de Trois-Rivières and Université de Montréal, Dr. J. Langlais, Montréal General Hospital, Canada, and Dr. D. Groleau, Biotechnology Research Institute, Montreal, Canada. This research was partly supported by grants from J. A. de Sève Foundation and from Medical Research Council, Canada, as well as PENCE.

**REFERENCES**


© 1999 Biochemical Society
Diterpine inhibitors of prohormone convertases

40 Jalal, M. F., Overton, K. H. and Rycroft, D. S. (1979) Phytochemistry 18, 149–156

Received 24 June 1998/28 October 1998; accepted 24 November 1998


© 1999 Biochemical Society