す。やがて、70年度の要領改訂により、新設の「基礎理科」という総合科目が登場して必修が6単位に半減、さらに82年度施行の要領では、必修は「基礎理科」に代わる総合科目「理科I」の4単位のみとなるのです。

94年度施行の要領では、「理科I」に代わる「総合理科」も選択科目になりました。つまり、必修単位が消滅し、理科4領域のうち、いくつかの分野については、まったく学ばなくても卒業が可能となったのです。その〝第一波〟が、97年度の入学組――私たちに補習授業を決意させた学生たちです。

そして、03年度施行の現行の要領では、「理科基礎」「理科総合A」「理科総合B」という新しい選択制の総合科目ができるとともに、以前は中学校で全員が学んだ生物の進化やイオンのはたらきなどが、高校の選択科目に移されることになりました。こうして、まったく学ばなくても卒業できる領域が増え、十分な基礎知識のないままに東大をはじめ、全国の大学に入学してくる学生が登場することになったのです。彼らが度重なる教育課程の「改悪」の犠牲者であることは、十分におわかりいただけると思います。

学びたい子が学べるアメリカ

さて、そこで生物教科書です。高校で生物をまったく学ばなくても生物関連分野の学部に進学できるという、現行の教育制度は大問題ですが、それでは高校時代に生物の授業を受けた子なら問題がないかというと、そうではありません。これがまた大問題なのです。「ゆとりの教育」の導入によって、高校では授業時間が大幅に削られただけでなく、自然科学にとって大事な「実験」も満足に行われず、いまやピペットの使い方もよくわからない学生が、大挙して進学してくる有り様です。

せめて教科書に授業内容の削減を補うぐらいの充実した内容があればと思うのですが、実際は充実どころか、おそろしく内容の乏しいものになっています。専門家の目からみても、21世紀を生きる人に必要な生物知識を学べる内容とはとうてい思えません。

私は2000年ごろから、機会があるたびに世界各国の「高校生物」の教科書を入手しては、日本の教科書との比較検討を行っています。アメリカのみならず、中国などと比較しても、日本の教科書は質・量ともに大きく後れているのが実態です。

現在までに私が検討を行った教科書は9ヵ国――日本、韓国、台湾、中国、アメリカ、イギリス、フランス、オランダ、チェコ――。まず、驚かされたのは、各国ごとのボリュームの違いでした。国ごとに冊数も判型もページ数も異なるため、文字や写真、図解などが印刷されている面積、すなわち「総印刷面積」で比較を行ったところ、以下のような結果となりました。アメリカ＝55（単位は㎡・以下同じ）、イギリス＝30、オランダ＝28・2、チェコ

＝20・5、中国＝16・7、台湾＝15・8、フランス＝14・5、韓国＝13・5。日本は10㎡で最下位でした。単純な言い方をすれば、日本では高校時代に10m×1m分の生物の知識しか学ぶ機会がないということです。

しかも、日本は10㎡の内容すべてが「選択」であり、前述のとおり、理系に進む生徒でさえ「0㎡」の子も少なくないのです。対して、他の国ではいずれも何割かが「必修」で、文系に進む生徒でもきちんと学びます。このリテラシー（理解力）の差は大きい。

日本の何倍ものボリュームがある欧米の教科書は、大学で学ぶような高度な内容も含んでいます。たとえばアメリカの場合、通常の高校の勉強を終えてしまった生徒で、在学中にさらに上級の勉強を希望する子は、同じ教科書を使って「AP（アドバンスト・プレイスメント）プログラム」という授業を受けることができます。この授業を選択して試験をパスすると、多くの大学でそれに相当する授業の単位が認められます。これは生物に限りません。

アメリカの教科書が多分に高度な内容を含んでいるのはそのためであり、「学びたい子」が「学べる環境」がきちんと用意されているのです。大学で高校の補習をせざるをえない日本とは大違いです。

間違いを教える教科書

他国の教科書を読み進めるうちに、ボリュームの少ない日本の教科書は、内容も相当乏しいことがわかり、私は暗鬱たる気持ちになりました。

たとえば、他の8ヵ国すべてが取り上げている大事な項目なのに、日本の教科書だけが取り上げていないものがいくつもありました。「コドン表」もそのひとつです。

生物はみな、DNA（デオキシリボ核酸）内にある「設計図」をもとにアミノ酸を合成しますが、その設計図はRNA（リボ核酸）に転写され、「A（アデニン）」、「U（ウラシル）」、「G（グアニン）」、「C（シトシン）」といった塩基4つのうちの3つの配列（コドン）で示されます。その塩基配列が、どのようなアミノ酸の合成を指定しているかを示した表がコドン表です。コドンが全生物に共通であることを学ぶことによって、地球上に存在するすべての生物が同じ起源を持っていることを科学的に理解することができます。生物の理解には欠かせないこのような大事なことが、日本の教科書には高校生全体の10％しか学ばない「生物II」の、しかもごく一部のみにしか載っていません。

「骨髄のはたらき」も、他の国々では必ず掲載しているのに、日本の教科書では取り上げていない項目です。骨髄移植や骨髄バンクの必要性を認識することは、これからの市民としてはきわめて大事なことで、そのためには、まず骨髄のはたらきを理解する必要があります。ところが、日本では01年の教科書検定で、「血液は骨髄でつくられる」という基本事項を説明した「生物

I」の記述が「不必要」とされ、削除されてしまったのです。

「遺伝」については、大事なことが記載されていないだけでなく、学ぶ順序についても、日本だけがおかしな順番になっているという問題があります。

遺伝を教えるには、まず「遺伝子」という物質的な実体があることを示し、次に個体レベルでの「遺伝の仕組み」を説き、そしてその応用として、遺伝子性疾患を含めた「人間の遺伝の問題」について教えるというのが、学問的に順序立った教え方です。当然ながら、他の8ヵ国の教科書ではそのような構成になっています。

ところが、日本ではまず、「生物I」で「メンデルの遺伝の法則」が登場し、つづいて「生物II」で遺伝子の実体について記されています。法則という仕組みが先で、その正体（実体）は後で学ぶという、まことにおかしなことになっているわけです。しかも、人間の遺伝の問題については、日本だけが取り上げていません。

教科書で「何」をどこまで取り上げるかについては国内の議論が絶えません。しかし、生物など理数科目の教科書は、歴史教科書などとは違って、国際比較によって、ある程度の答えを得ることが可能なはずです。いまの日本の生物教科書は、世界の生物教育の趨勢から大きく逸脱していると言わざるをえません。

専門家として許せないのは、本来教えるべき内容を無理に削ったその〝辻褄あわせ〟のために、明らかな間違いを、さも正しいかのように教える記述があることです。たとえば「イオン」に関する記述です。

こと生物においては、細胞の外側には電荷を帯びたナトリウムイオン、同じく内側にはカリウムイオンが多く、その濃度勾配によってさまざまな生化学現象が生まれています。ところが、現行の教科書は「ナトリウムの濃度」

「カリウムの濃度」と書いてあります。一見些細なことのように思えますが、これはとんでもない間違いです。

化学の世界で「ナトリウム」とは「金属ナトリウム」のことであり、水分に触れると爆発的に燃焼する性質を持っているものです。「カリウム」についてもほぼ同様です。細胞内に存在するはずがないのです。なぜ、そのような馬鹿げた記述になるのでしょうか。

前述のとおり、03年度施行の学習指導要領で、従来は中学の理科で全員が習っていたイオンの項目が、高校の選択科目に移行しました。そのため、各社の高校生物の教科書は、当初はイオンについての補助解説をつけたうえで細胞内外の生化学現象を記述しました。ところが検定の結果、「生物の教科書ではイオンの説明は不要」とし、「ナトリウムイオン」は「ナトリウム」でよし、としたのです。真実よりも制度の都合を優先させるという、笑うに笑えない事態となっているわけです。

「人間」の視点が欠落

日本の教科書にはさらに、生物を教える「姿勢」の問題もあると私は考えています。つまり、「なぜ、子どもたちに『生物』を教えるのか」という問題です。多くの国々の教科書では、「生物教育」を、最終的に人間、すなわち生徒自身や身近な人々の健康や安全の問題として関連づけていく「ヒューマン・バイオロジー」の姿勢が顕著です。

たとえば、日本の教科書には「人間の妊娠出産」という項目が完全に欠落しています。ウニやカエルにおける古典的な「発生」にとどめて、ヒトの発生を扱わないのは日本の教科書だけです。

高校生といえば、性への関心が一番旺盛な世代です。どこまで記述するかという点で議論の余地はあるにせよ、妊娠出産の問題をとおして、子どもたちの「知りたい」という気持ちに、生物学的立場から真摯に応えようという姿勢が世界各国の教科書にはあります。生物学者を目指すわけではない大多数の生徒にも、生物を学ぶ意味が自然に伝わる内容になっています。

日本の場合、「人間」については保健で多くを教えることになりますが、そこでの人間の扱い方は、科学的というよりも社会学的で、それも「健全な社会を保つために、あれをしてはいけない、これをしてはいけない」という指導書的な記述が目立ちます。それに先立つ「生物としてのヒト」という視点からの教育が、我が国には保健にも生物にも欠けていると思います。

ヒューマン・バイオロジーの観点から、具体的にどのような問題をどこまで大きく取り上げるかは、国によってまちまちです。オランダやフランスの教科書は、ヒトの誕生のところで、避妊具の使い方まで図版入りで教えています。生物の授業でこうした問題を取り扱うことは、中絶や性感染症といった問題に対する大人からの「真面目な

メッセージ」でもあるのです。

アメリカの教科書は、喫煙の危険性や薬物乱用が人体に及ぼす影響などを取り上げている点に〝お国の事情〟が感じられます。遺伝子の突然変異と発がんを扱った章で、喫煙による肺がんの危険性が写真と解説で掲載されていますし、薬物乱用については神経伝達を扱った章で、やはり図解入りで詳しく取り上げられています。ただし、指導書的な物言いではなく、薬物によって神経伝達物質のはたらきがどう変化し、神経系や脳のシステムを崩壊させるかといった、あくまでも生体のメカニズムについての記述が中心です。

中国の教科書では、遺伝について学ぶ章のところで、ダウン症や筋ジストロフィーなど、人間の遺伝子性疾患について挙げています。他の国の遺伝子性疾患についての記述よりも、多くのスペースを割いています。

遺伝子性疾患は、生物学的には人口の一定割合で生じる自然現象で、完全に防ぐことはできません。だから、社会的には、そのことをよく理解して、「患者のケアは社会でになう」というコンセンサスを築く必要があります。

ところが、日本ではショウジョウバエの遺伝と突然変異について教えるにとどまり、人間の遺伝子性疾患についてはほとんど教えていません。大学の教養課程の生物の授業ですら、教えていないのが現実です。他の先進国と比べ、患者を産んだ母親が責められたり、家族が負担を背負うのが当たり前とされたりする風潮が、今日にいたるまで根強く残っているのは、生物教育と無関係ではないと私は思います。

「国際生物学オリンピック」（IBO）という組織が、04年に「高校生物教育のカリキュラムの中で、植物・動物・人間・遺伝などの各テーマが、どの程度の割合で扱われているか」について調査を行い、加盟国の平均を公表したことがあります。国際平均値では、全

部で10あるテーマの中で「人間」の割合が全体の19%を占め、もっとも重点的な教育が施されています。まさに、ヒューマン・バイオロジーは世界の主流といってもよいでしょう。

ちなみに、日本の平均値では「人間」はわずかに4%。全テーマの中で国際平均から一番かけ離れています。子どもたちの生物・理科教育離れの傾向が止まらないのは、いつまでもウニやショウジョウバエで生命の仕組みを学び「人間」の問題として捉えにくいところがあるからではないでしょうか。

本当の"ミニマム"とは

昨年、私はある中国人女性の知人から、「日本の教科書のレベルの低さにビックリした」と言われました。彼女は日本人の夫との間に生まれた子どもを中国で育てていたのですが、夫の仕事の関係で日本で暮らすことになり、子どもがもらってきた教科書を見て失望したというのです。そしていま、このまま何年も日本の学校で学ぶと、もう中国には子どもを戻せなくなると、本気で心配しています。

彼女から入手した教科書を見て、私は最新の生物教育のエッセンスが、ほどよい分量で詰まった中国の教科書のその完成度の高さに驚きました。恥ずかしい話ですが、それまでは、中国の理数教育のレベルの高さをよく知らなかったのです。聞けば、中国は、かつての文化大革命で崩壊した教育制度を欧米から戻ってきた留学者たちが立て直したということです。たしかに、中国の教科書はアメリカのそれと非常に似通っており、英訳すれば英語圏でも十分に通用するだろうと思います。もし、9ヵ国の教科書の中から、私が「日本で採用したい教科書」を1冊選べるとしたら、迷わず中国の教科書を選ぶと思います。

すでに指摘されはじめていますが、日本人一般の「科学リテラシー」の低さが問題になっています。OECD（経済協力開発機構）が加盟国の成人を対象に行う科学知識の調査がありますが、91年に行われた最初の調査では、日本は14ヵ国中13位。10年後の01年の調査でも15ヵ国中13位という結果です。

国内の識者の中には、こういった調査結果を引き合いに出して「理科の4領域を学んだ世代がいてもこの程度の状態なのだから、2領域に絞って勉強させたほうが効果的だ」という趣旨の発言を行う人がいます。しかし、科学リテラシーとは、トータルな科学の知識が問われているのですから、2領域しか勉強しなくなった世代で科学リテラシーが高まるとは思えません。

文部科学省は、現在の教科書の内容や科目の選択のことを「ミニマム・エッセンス」と位置づけています。選択によって生物知識がゼロになったり、化学知識がゼロになったりするような子どもが増えるような状況をミニマム・エッセンスと呼ぶのは、明らかに間違いです。生物も化学も物理も地学も、そ

れぞれに学ぶべきミニマムがあり、その総合が求められる科学リテラシーのミニマムであると、私は考えます。

環境ホルモン、ゲノム情報、地球温暖化、地震対策、人口調節――21世紀の時代は科学リテラシーがますます求められていく時代になるはずです。現行のような、お寒いかぎりの理科教育で来たるべき時代に備えられるでしょうか。子どもたちのために、私たちは何ができるでしょうか。その意味で、現代に生きる人間の問題をきちんと取り上げ、真摯に取り組む諸外国の生物教科書に、私は大人たちの「親心」を感じました。残念ながら、日本の教科書にはそれが感じられないのです。

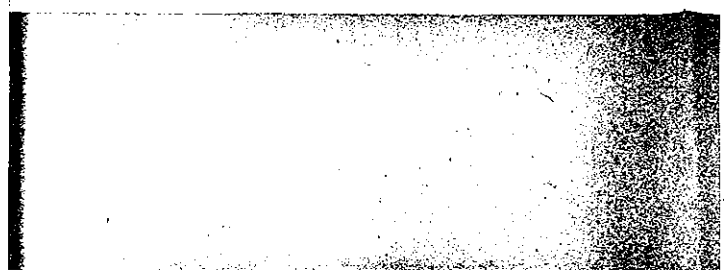
いま、私の手元に、つい最近入手したばかりのニュージーランドの高校生物の教科書があります。最初の案内文には、アメリカのAPプログラムや、「インターナショナル・バカロレア」という国際的な大学入学資格取得プログラムにも対応した教科書であると書かれています。自国だけでなく英語圏をマーケットと考え、世界で販売できるような生物教育の最高水準を見据えた教科書づくりをしているのです。

繰り返しになりますが、日本はこのままでは、先進国はおろか発展途上国よりも科学リテラシーの低い国になりかねません。これは決して大袈裟な言い方ではないのです。急成長する途上国の多くは、世界水準の教育で先進国に追いつき追い越そうとしているからです。その好例が中国です。

日本は国際競争力維持のためにも、そして、子どもたち個々人が自分の生活を守れるだけのミニマムの科学リテラシーを身につけるためにも、世界と比肩できるだけの内容の教科書を持つべきなのではないでしょうか。

取材協力・中和正彦

まつだ・りょういち　52年生まれ。東京都立大学助手などを経て91年より現職。共著に『どうする「理数力」崩壊』。「高等教育フォーラム」代表



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Tripropeptins, Novel Antimicrobial Agents Produced by *Lysobacter* sp.

II. Structure Elucidation

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Planar structures of tripropeptins (TPPs) were elucidated by spectroscopic studies including various NMR measurements. Stereochemistry of constituent amino acids of tripropeptin C (TPPC) (**3**) was identified by marfey's method except hydroxyproline which was determined by studies of NMR and CD spectra. The absolute structure of **3** was determined by analyses of the fragments obtained by Birch reduction and $LiBH_4$ reduction of **3**. The configuration of the fatty acid, isolated from acid hydrolysate of **3**, was determined to be (3*R*)-hydroxy-13-methyltetradecanoic acid from MS, NMR spectra and negative sign of the optical rotation.

We have isolated tripropetin A (**1**), B (**2**), C (**3**), D (**4**) and Z (**5**) (Fig. 1), as antimicrobial agents, from cultured cells and broth of *Lysobacter* sp. designated BMK333-48F3. In the preceding paper, the taxonomy, isolation and biological activities were reported[1]. In this paper, we describe the physico-chemical properties and structure determination of TPPs.

Result and Discussion

Tripropeptin C (**3**), a main component of TPPs, was isolated as colorless powder and its UV spectrum showed end absorption. **3** gave positive color reaction with iodide vapor, Rydon-Smith and Sakaguchi reagent. IR spectrum of **3** showed characteristic absorption of peptide bonds (1635 and 1537 cm^{-1}) and of lactone linkage (1737 cm^{-1}). Molecular formula for **3** was determined by HRFAB-MS as $C_{51}H_{83}N_{11}O_{19}$ (calcd. 1154.5927 for $(M+H)^+$, found 1154.5945), which was supported by the ^1H and ^{13}C NMR spectral data.

Other tripropeptins showed similar results, as summarized in Table 1 and these properties suggested that every tripropeptin belongs to depsipeptide antibiotics.

Planar structure of **3** was determined as follows. All bond connections between ^1H and ^{13}C signals were interpreted by DEPT and heteronuclear multiple quantum coherence (HMQC) experiments. The DEPT and HMQC experiments revealed the presence of three methyl, twentytwo methylene, fourteen methine, one sp^2 quaternary and eleven carbonyl carbons in **3**. The ^1H and ^{13}C NMR spectral data of **3** are shown in Table 2. The ^1H-^1H COSY and HMBC spectra of **3** indicated the presence of β-hydroxy fatty acid and eight amino acids, threonine (Thr), serine (Ser), arginine (Arg) and hydroxyproline (OHPro), one residue each, and 2 residues of proline (Pro) and β-hydroxyaspartic acid (β-OHAsp) in Fig. 2.

The sequence of **3** was determined by HMBC spectrum as follows. The correlation from H-2 (δ 4.62) of β-OHAsp (II) to carbonyl carbon C-5 (δ 169.9) of OHPro, from H-6

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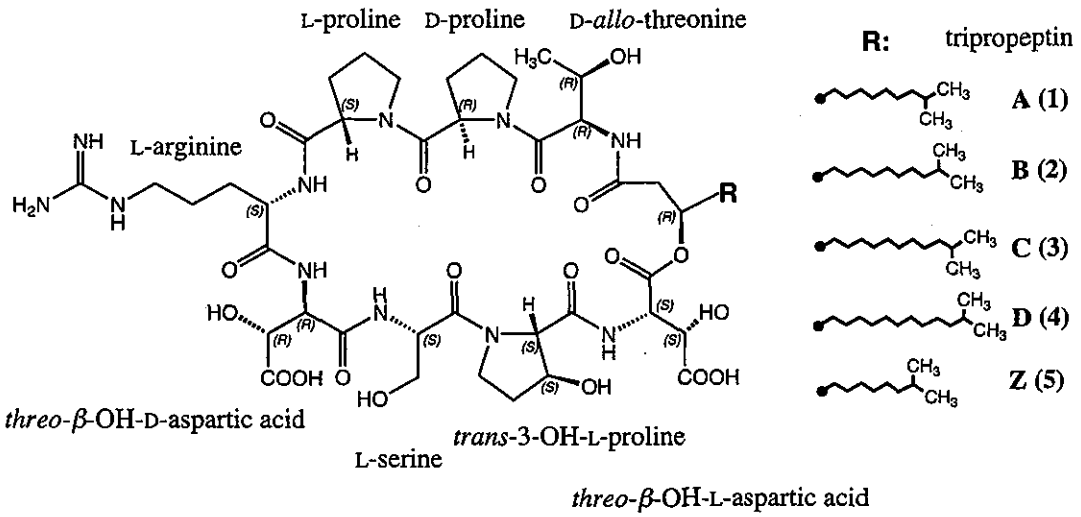
Table 1. Physicochemical properties of tripropeptin A, B, C, D and Z.

	A	B	C	D	Z
$[\alpha]_D^{24}$ (MeOH)	-7.8° (*c* 1)	-7.9° (*c* 1)	-8.4° (*c* 1)	-10.8° (*c* 1)	-14.0° (*c* 1)
HRFAB-MS(*m/z*)					
found	1126.5657(M+H)$^+$	1140.5788(M+H)$^+$	1154.5945(M+H)$^+$	1168.6101(M+H)$^+$	1112.5475(M+H)$^+$
Calcd.	1126.5632	1140.5776	1154.5927	1168.6074	1112.5491
Molecular formula	$C_{49}H_{79}N_{11}O_{19}$	$C_{50}H_{81}N_{11}O_{19}$	$C_{51}H_{83}N_{11}O_{19}$	$C_{52}H_{85}N_{11}O_{19}$	$C_{48}H_{77}N_{11}O_{19}$
IR υ_{max}(KBr)cm^{-1}	3375, 2923, 1737, 1635, 1538, 1450, 1263, 1203, 1097	3345, 2931, 1737, 1635, 1537, 1450, 1263, 1201, 1097	3372, 2927, 1737, 1635, 1537, 1452, 1263, 1203, 1097	3282, 2931, 1739, 1633,1537, 1452, 1263,1203,1099	3388, 2923, 1725, 1635, 1536, 1450, 1265, 1205,1095
TLC, Rf value[a]					
BuOH-MeOH-H_2O(4:1:2)	0.45	0.45	0.45	0.45	0.45
$CHCl_3$-MeOH-H_2O(10:5:1)	0.25	0.25	0.25	0.25	0.25
Color Reaction					
positive	Rydon-Smith, Sakaguchi	Rydon-Smith, Sakaguchi	Rydon-Smith, Sakaguchi	Rydon-Smith, Sakaguchi	Rydon-Smith, Sakaguchi
Soluble	MeOH,DMSO,H_2O	MeOH,DMSO,H_2O	MeOH,DMSO,H_2O	MeOH,DMSO,H_2O	MeOH,DMSO,H_2O
Insoluble	$CHCl_3$,acetone,EtOAc	$CHCl_3$,acetone,EtOAc	$CHCl_3$,acetone,EtOAc	$CHCl_3$,acetone,EtOAc	$CHCl_3$,acetone,EtOAc

[a] Merck Kieselgel 60F_{254} Art. 5715

Fig. 1. Structure of tripropeptins.



(δ 4.23) of OHPro to carbonyl carbon C-10 (δ 167.9) of Ser, from an amide proton (δ 7.25) of Ser to carbonyl carbon C-13 (δ 168.5) of β-OHAsp (I), from an amide proton (δ 8.49) of β-OHAsp (I) to carbonyl carbon C-17 (δ 171.2) of Arg, from an amide proton (δ 7.77) of Arg to carbonyl carbon C-23 (δ 172.6) of Pro (I), from H-27 (δ 3.35) of Pro (I) to carbonyl carbon C-28 (δ 172.0) of Pro (II), from H-29 (δ 4.18) of Pro (II) to carbonyl carbon C-33 (δ 169.0) of Thr, from methine protons H-34 (δ 4.52) and H-39 (δ 5.06) to carbonyl carbon C-37 (δ 169.5) of 3-hydroxy-13-methyltetradecanoic acid indicated that the sequence of **3** to be 3-hydroxy-13-methyltetradecanoyl-Thr-Pro-Pro-Arg-β-OHAsp-Ser-OHPro-β-OHAsp.

A long-range coupling between C-1 (δ 168.6) and H-39

Table 2. ^{13}C and ^{1}H NMR data of tripropeptin C in DMSO-d_6.

position	type	δ C[a]	δ H[b](multiplicity, J (Hz))
1	>C=O	168.6	
2	>CH-N	54.8	H:4.62(1H, m), NH:7.80(1H, d, 10.0)
3	>CH-O	70.0	4.55(1H, d, 2.4)
4	>C=O	171.8	
5	>C=O	169.9	
6	>CH-N	68.8	4.23(1H, s)
7	>CH-O	72.5	4.26(1H, d, 3.8)
8	-CH_2-	32.3	1.75(2H, m)
9	-CH_2N<	45.0	3.53(1H, m), 3.66(1H, m)
10	>C=O	167.9	
11	>CHNH-	53.1	H:4.58(1H, m), NH:7.25(1H, d, 8.0)
12	-CH_2O-	61.3	3.53(2H, m)
13	>C=O	168.5	
14	>CHNH-	56.3	H:4.64(1H, m), NH:8.49(1H, d, 8.4)
15	>CH-O	70.0	4.5(1H, d, 2.0)
16	>C=O	172.9	
17	>C=O	171.2	
18	>CHNH-	51.7	H:4.55(1H, m), NH:7.77(1H, d, 8.6)
19	-CH_2-	28.9	1.56(1H, m), 1.63(1H, m)
20	-CH_2-	24.7	1.35(2H, m)
21	-CH_2NH-	40.3	H:3.06(2H, m), NH:7.62(m)
22	-N=C(N-)N-	156.9	
23	>C=O	172.6	
24	>CH-N	60.6	4.72(1H, m)
25	-CH_2-	31.7	1.93(1H, m), 2.13(1H, m)
26	-CH_2-	22.2	1.78(2H, m)
27	-CH_2-N	46.9	3.35(1H, m), 3.49(1H, m)
28	>C=O	172.0	
29	>CH-N	57.9	4.18(1H, t, 12.4)
30	-CH_2-	29.0	1.63(2H, m)
31	-CH_2-	24.4	1.75(1H, m), 1.85(1H, m)
32	-CH_2-N	47.3	3.54(1H, m), 3.61(1H, m)
33	>C=O	169.0	
34	>CHNH-	56.0	H:4.52(1H, d, 7.0), NH:8.04(1H, d, 8.4)
35	>CH-O	67.2	3.74(1H, m)
36	-CH_3	19.0	0.97(3H, d, 6.6)
37	>C=O	169.5	
38	-CH_2-	40.1	2.28(1H, d, 12.0), 2.66(1H,m)
39	>CH-O	72.8	5.06(1H, m)
40	-CH_2-	33.7	1.50(2H, m)
41	-CH_2-	24.1	1.21(2H, m)
42	-CH_2-	29.1	1.21(2H, m)
43	-CH_2-	29.1	1.21(2H, m)
44	-CH_2-	29.1	1.21(2H, m)
45	-CH_2-	29.1	1.21(2H, m)
46	-CH_2-	29.1	1.21(2H, m)
47	-CH_2-	26.8	1.13(1H, m), 1.21(1H, m)
48	-CH_2-	38.5	1.13(1H, m), 1.21(1H, m)
49	>CH-	27.4	1.49(1H, m)
50	-CH_3	22.5	0.83(3H, d, 7.0)
51	-CH_3	22.5	0.83(3H, d, 7.0)

[a] 125 MHz, chemical shift in ppm.
[b] 500 MHz, chemical shift in ppm.

(δ 5.06) was observed by decoupled HMBC[2]. This clearly indicated the lactone linkage forming between acyl chain and β-OHAsp (II). According to these data, planar structure of 3 was determined as shown in Fig. 2. Planar structures of other compounds, **1**, **2**, **4** and **5** were determined likewise. The ^{13}C NMR spectral data of **1**, **2**, **3**, **4**, **5** are shown in Table 3.

The stereochemistry of constituent amino acids were determined using Marfey's method[3] except hydroxyproline. Hydrolysis of **3** and its degradation products **6** and **7** gave the corresponding amino acids. The acid hydrolysates were converted to Marfey's derivatives by treating with 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (L-FDAA), and analyzed by HPLC. Each amino acid derivatives was identified by comparing the retention time with that of the Marfey's derivatives of authentic amino acid. The Marfey's derivatives of amino acids liberated from **3** showed peaks matching L-arginine (L-Arg), L-serine (L-Ser), D-*allo*-threonine (D-*a*Thr), *threo*-β-hydroxy-L-aspartic acid (*threo*-β-L-OHAsp), *threo*-β-hydroxy-D-aspartic acid (*threo*-β-D-OHAsp), L-proline (L-Pro) and D-proline (D-Pro).

The positions of D,L-proline and D,L-hydroxyaspartic acid were determined as follows. Amino acid analysis of the **6**, obtained by Birch reduction[4] of **3**, showed **6** comprising L-Arg, L-Ser, *threo*-β-L-OHAsp, *threo*-β-D-OHAsp, L-Pro (Fig. 3), indicated partial amino acid sequence of L-Pro-L-Arg. The amino acid analysis of **7**, obtained by $LiBH_4$ reduction[5] of **3**, showed **7** comprising L-Arg, L-Ser, D-*a*Thr, *threo*-β-D-OHAsp, L-Pro and D-Pro (Fig. 3), indicated hydroxyaspartic acid forming lactone linkage was *threo*-β-L-OHAsp.

The absolute structure of hydroxyproline (**8**) was identified to be L-*trans*-3-hydroxyproline[6] by the plus cotton effect at 220 nm in the CD spectrum [CD; $[\theta]_{240}$ +80, $[\theta]_{220}$ +1980, $[\theta]_{210}$ +2980 (*c* 0.033, 0.5 M HCl)] and the small coupling constant between H-2 and H-3 in ^{1}H NMR spectrum ($J_{2,3}$=1.60 Hz), in the literature [*trans* configuration, $J_{2,3}$=1.2 Hz and *cis* configuration, $J_{2,3}$=4.2 Hz].

The absolute configuration of the fatty acid (**9**) was determined to be (3*R*)-hydroxy-13-methyltetradecanoic acid (Fig. 4) from MS, NMR and negative sign of the optical rotation[5,7] $[\alpha]_D^{24}$ −7.7° (*c* 0.13, $CHCl_3$), in the literature $[\alpha]_D^{20}$ −12.7° (*c* 0.14, $CDCl_3$)[5].

According to these data, the absolute structure of **3** is determined as shown in Fig. 1.

Experimental

General

Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. UV spectra were determined on a Hitachi 557 spectrophotometer. IR spectra were recorded

Fig. 2. ^{1}H-^{1}H COSY and HMBC experiments of tripropeptin C in DMSO-d_6.

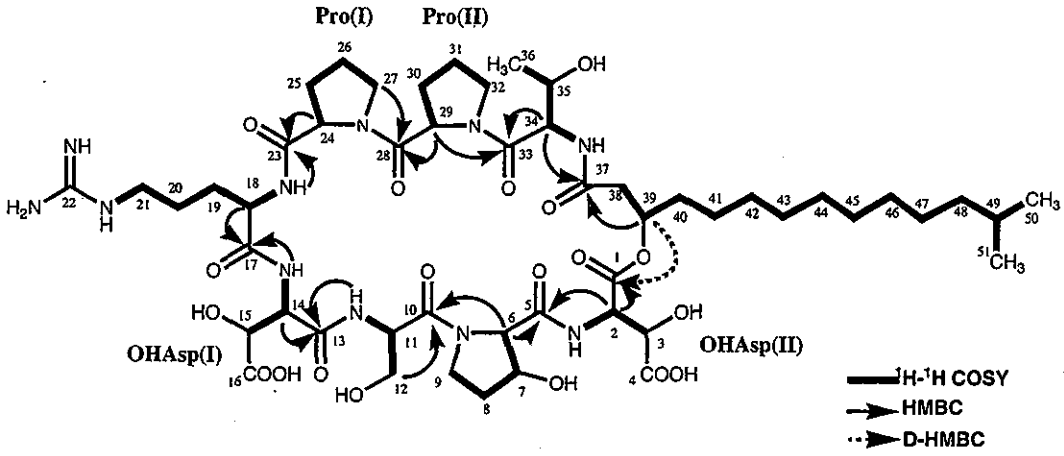


Table 3. ^{13}C NMR data of tripropeptins.

	δ c* (multiplicity) DMSO-d_6				
position	A	B	C	D	Z
1	169.7 (s)	169.8 (s)	168.6 (s)	168.6 (s)	170.2 (s)
2	53.0 (d)	54.9 (d)	54.8 (d)	54.8 (d)	55.1 (d)
3	69.9 (d)	70.3 (d)	70.0 (d)	70.0 (d)	70.6 (d)
4	172.7 (s)	172.6 (s)	171.8 (s)	171.9 (s)	172.7 (s)
5	171.3 (s)	171.5 (s)	169.9 (s)	169.9 (s)	171.5 (s)
6	66.4 (d)	67.2 (d)	68.8 (d)	68.8 (d)	67.2 (d)
7	70.0 (d)	70.4 (d)	72.5 (d)	72.5 (d)	70.8 (d)
8	32.4 (t)	31.8 (t)	32.3 (t)	32.3 (t)	32.8 (t)
9	44.8 (t)	44.1 (t)	45.0 (t)	45.0 (t)	44.1 (t)
10	168.8 (s)	168.4 (s)	167.9 (s)	167.9 (s)	168.4 (s)
11	52.9 (d)	52.5 (d)	53.1 (d)	53.1 (d)	52.5 (d)
12	60.5 (t)	60.1 (t)	61.3 (t)	61.3 (t)	60.5 (t)
13	169.0 (s)	169.5 (s)	168.5 (s)	168.5 (s)	169.9 (s)
14	56.1 (d)	57.3 (d)	56.3 (d)	56.3 (d)	57.7 (d)
15	66.9 (d)	70.0 (d)	70.0 (d)	70.0 (d)	69.4 (d)
16	172.9 (s)	174.3 (s)	172.9 (s)	172.9 (s)	174.9 (s)
17	172.0 (s)	171.6 (s)	171.2 (s)	171.2 (s)	170.2 (s)
18	51.8 (d)	52.1 (d)	51.7 (d)	51.7 (d)	52.1 (d)
19	28.6 (t)	29.1 (t)	28.9 (t)	28.9 (t)	29.0 (t)
20	24.6 (t)	25.2 (t)	24.7 (t)	24.7 (t)	24.9 (t)
21	39.5 (t)	39.5 (t)	40.3 (t)	40.3 (t)	40.4 (t)
22	156.7 (s)	156.6 (s)	156.9 (s)	156.9 (s)	156.7 (s)
23	172.9 (s)	174.2 (s)	172.6 (s)	172.6 (s)	174.3 (s)
24	58.0 (d)	59.9 (d)	60.6 (d)	60.6 (d)	60.1 (d)
25	31.7 (t)	31.4 (t)	31.7 (t)	31.7 (t)	31.9 (t)
26	22.3 (t)	22.2 (t)	22.2 (t)	22.2 (t)	22.3 (t)
27	44.8 (t)	47.0 (t)	46.9 (t)	46.9 (t)	47.1 (t)
28	172.2 (s)	172.9 (s)	172.0 (s)	172.0 (s)	173.1 (s)
29	56.4 (d)	57.8 (d)	57.9 (d)	57.9 (d)	57.9 (d)
30	27.5 (t)	28.8 (t)	29.0 (t)	28.4 (t)	28.9 (t)
31	24.5 (t)	24.8 (t)	24.4 (t)	24.4 (t)	24.7 (t)
32	47.0 (t)	47.3 (t)	47.3 (t)	47.3 (t)	47.4 (t)
33	169.7 (s)	169.9 (s)	169.0 (s)	169.0 (s)	170.4 (s)
34	54.8 (d)	56.0 (d)	56.0 (d)	56.0 (d)	56.0 (d)
35	65.9 (d)	65.8 (d)	67.2 (d)	67.2 (d)	65.9 (d)
36	18.9 (q)	18.6 (q)	19.0 (q)	19.0 (q)	18.6 (q)
37	169.7 (s)	170.3 (s)	169.5 (s)	169.6 (s)	170.5 (s)
38	38.6 (t)	38.1 (t)	40.1 (t)	39.5 (t)	39.5 (t)
39	73.0 (d)	73.1 (d)	72.8 (d)	72.8 (d)	73.1 (d)
40	33.6 (t)	33.0 (t)	33.7 (t)	33.7 (t)	33.0 (t)
41	24.0 (t)	23.6 (t)	24.1 (t)	24.1 (t)	23.4 (t)
42	29.0 (t)#	29.1 (t)#	29.1 (t)#	29.1 (t)#	29.2 (t)#
43	29.0 (t)#	29.2 (t)#	29.1 (t)#	29.1 (t)#	29.3 (t)#
44	29.1 (t)#	29.4 (t)#	29.1 (t)#	29.0 (t)#	26.8 (t)
45	24.7 (t)	29.2 (t)#	29.1 (t)#	28.7 (t)#	38.7 (t)
46	38.6 (t)	26.9 (t)	29.1 (t)#	29.2 (t)#	27.6 (d)
47	26.9 (d)	38.1 (t)	26.8 (t)	29.3 (t)#	22.7 (q)
48	22.6 (q)	27.5 (d)	38.5 (t)	26.8 (t)	22.7 (q)
49	22.6 (q)	22.7 (q)	27.4 (d)	38.5 (t)	-
50	-	22.7 (q)	22.5 (q)	27.4 (d)	-
51	-	-	22.5 (q)	22.5 (q)	-
52	-	-	-	22.5 (q)	-

* 125 MHz, chemical shift in ppm.
# undistinguishable

Fig. 3. Amino acid analysis of the fragments **6** and **7**.

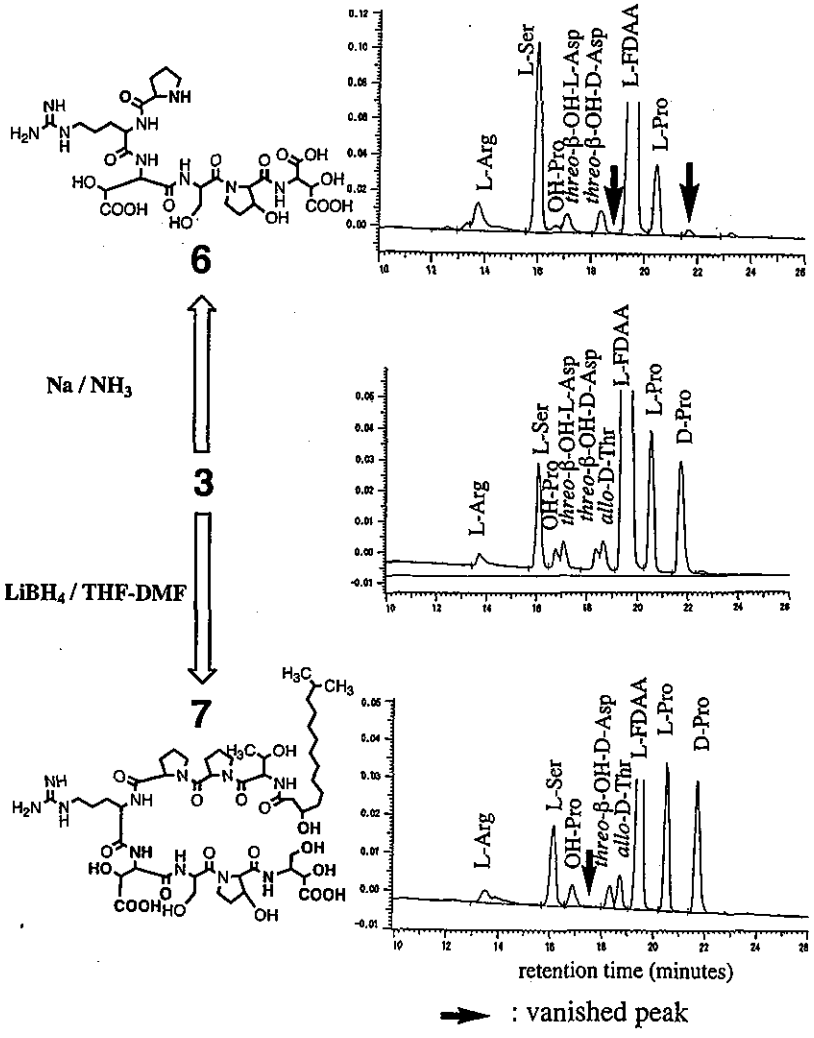
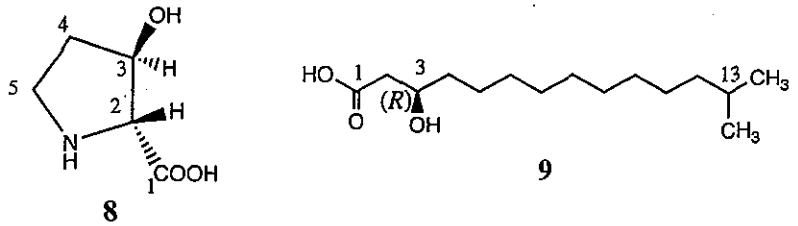


Fig. 4. L-*trans*-3-Hydroxyproline (**8**) and 3-hydroxy-13-Me-tetradecanoic acid (**9**).



using a Horiba FT-210 fourier transform infrared spectrometer. Mass spectra were recorded using a HITACHI M1200H LC/MS (APCI), JEOL JMS-SX102 (HRFAB) and JEOL JMS-T100LC (HRESI) mass spectrometer. The NMR spectra were measured using a JEOL JNM-A500 spectrometer. CD spectrum was recorded using a JASCO J-720W spectropolarimeter.

Materials

L-Arginine (L-Arg), L-serine (L-Ser), L-threonine (L-Thr),

L-proline (L-Pro), D-serine (D-Ser), D-threonine (D-Thr), D-proline (D-Pro) and *erythro*-β-hydroxy-L-aspartic acid (*erythro*-β-L-OHAsp) were purchased from WAKO Pure Chemical Industries, Ltd. D-Arginine (D-Arg) was purchased from SIGMA. L-3-Hydroxyproline (L-OHPro), *threo*-β-hydroxy-aspartic acid (*threo*-β-OHAsp), D-*threo*-β-hydroxyaspartic acid (*threo*-β-D-OHAsp), DL-*allo*-threonine (DL-*a*Thr), L-*allo*-threonine (L-*a*Thr) and 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (L-FDAA) were purchased from TOKYO KASEI.

Amino Acid Analysis using Marfey's Method

In a micro test tube, 0.5 mg of amino acid or acid hydrolysate of tripropeptin, was dissolved in 50 μl of H_2O, then, 20 μl of 1 M $NaHCO_3$ aqueous solution and 20 μl of 1% L-FDAA acetone solution were added. This reaction mixture was capped and incubated at 37°C for 60 minutes. After the addition of 20 μl of 1 M HCl aqueous solution into the tube to stop the reaction, the reaction mixture was evaporated to dryness. The residue, Marfey derivative, was dissolved in 1 ml of methanol and then 10 μl aliquot of the solution was injected into HPLC system. The analyses were performed on a ODS column (Capcell Pak, UG120, Shiseido 5 μm, 150×4.6 mm i.d.) using acetonitrile-0.01 M TFA aqueous solution as the mobile phase in the gradient elution mode (acetonitrile, 10%~40%, 30 minutes). The flow rate of the mobile phase was 2.0 ml/minute and the monitoring wavelength was set at 340 nm.

Purification of Constituent Amino Acids

40 mg of acid hydrolysate of **3** was dissolved in 1 ml of H_2O then added equal volume of ethylacetate. Ethylacetate layer was evaporated to dryness and was used for fatty acid analysis. Water layer was dried up, then subjected to column chromatography using 8 ml wet volume of AMBERLITE CG50I (NH_4^+ type resin, ROAM AND HAAS), eluted successively with 25 ml each of H_2O and 1 M NH_4OH. Amino acid, eluent and their dry weight (in parentheses) are as follows: OHAsp and Thr (H_2O, 3.8 mg), Ser (H_2O, 2.4 mg), Thr, OHPro and Pro (H_2O, 3.0 mg), Pro (H_2O, 4.0 mg), Arg (1 M NH_4OH, 1.1 mg). The former mixture was further chromatographed by using 20 ml wet volume of AMBERLITE CG50I (NH_4^+ type resin, ROAM AND HAAS) eluted with H_2O gave 2.1 mg of OHAsp and 0.8 mg of Thr. The latter mixture was also further chromatographed by using 20 ml wet volume of microcrystalline cellulose (FUNACEL, Funakoshi, Ltd.), eluted with stepwise gradient of acetone : H_2O (60 ml each of 16 : 4, 15 : 5, 14 : 6, 13 : 7). Fractions, eluted with acetone : H_2O (15 : 5), were dried up, then chromatographed by using 15 ml wet volume of microcrystalline cellulose, eluted with acetonitrile : H_2O (100 ml each of 88 : 12, 85 : 15). Eluted with latter solvent gave 1.3 mg of OHPro.

Stereochemistry of Hydroxyproline (**8**)

Hydroxyproline was obtained as colorless powder. FAB-MS; *m/z* 132.09 $(M+H)^+$, CD; $[\theta]_{240}$ +80, $[\theta]_{220}$ +1980, $[\theta]_{210}$ +2980 (*c* 0.033, 0.5 M HCl). ^1H NMR in D_2O at 10°C; δ 2.06 (m, 2H, H-4), 3.52 (m, 1H, H-5), 3.63 (m, 1H, H-5), 4.14 (d, J=1.60 Hz, 1H, H-2), 4.72 (m, 1H, H-3). ^{13}C NMR in D_2O at 10°C; δ 32.9 (C-4), 45.0 (C-5), 69.3 (C-2), 74.5 (C-3), 172.0 (C-1).

HPLC Analysis of the Marfey's Derivatives

Retention time (minutes) of standard amino acids-Marfey's derivatives were as follows: L-Arg (13.89), D-Arg (14.37), L-Ser (16.10), D-Ser (16.91), L-Thr (17.01), D-Thr (19.89), L-*a*Thr (16.75), D-*a*Thr (18.51), *threo*-β-L-OHAsp (17.20), *threo*-β-D-OHAsp (18.46), *erythro*-β-L-OHAsp (18.02), *erythro*-β-D-OHAsp (18.86), L-Pro (20.53), D-Pro (21.60). Retention time (minute) of the amino acids, isolated from acid hydrolysate of **3**, were as follows: Arg (13.89), Ser (16.08), Thr (18.51) , OHAsp (17.12, 18.40), Pro (20.48, 21.63).

Birch Reduction of **3**

The reduction was performed on 37.0 mg of tripropeptin C in 30 ml of liquid ammonia using 450 mg of sodium at −30°C. After 5 minutes, the reaction was terminated by the addition of 2.5 g of ammonium acetate, then concentrated. The residue was diluted with 30 ml of H_2O, then subjected to column chromatography using 80 ml wet volume of Dowex (50w×2, H^+ type, THE DOW CHEMICAL COMPANY) washed with 240 ml of H_2O and eluted with 240 ml of 1 M NH_4OH. The eluent was concentrated *in vacuo* then dissolved in small volume of H_2O and applied to HP20 column (Mitsubishi Chemical Co., 10 ml wet volume). The column was washed with 30 ml of deionized water, 30 ml of 50% aqueous methanol and acetone. Fractions eluted with H_2O gave 21.7 mg of **6** as colorless powder. HRESI-MS *m/z* 732.2844 $(M-H)^-$ (calcd. 732.2800 for $C_{27}H_{42}N_9O_{15}$).

Reduction of **3** with $LiBH_4$

2 mg of $LiBH_4$ was added to a solution of 10 mg of **3** in 50 μl of DMF and 2 ml of THF. The reaction mixture was refluxed for 4 hours. After the reaction mixture was cooled to room temperature, the solution was neutralized by 1 M HCl, then evaporated *in vacuo*. The residue was subjected to column chromatography using 20 ml wet volume of

Sephadex LH-20 (Pharmacia) eluted with methanol. Further purification by HPLC (Capcell Pak, UG120, Shiseido 5 μm, 150×4.6 mm i.d., the flow rate of the mobile phase was 2.0 ml/minute and the monitoring wavelength was set at 210 nm) using 35% acetonitrile aqueous solution as the mobile phase gave 7.1 mg of **7** as colorless powder. HRESI-MS *m/z* 1158.6422 $(M+H)^+$ (calcd. 1158.6362 for $C_{51}H_{87}N_{11}O_{19}$). IR (KBr); 3438, 2927, 1677, 1635, 1384, 1207, 1182, 1133 cm^{-1}.

Isolation and Configuration of Fatty Acid (**9**)

6.2 mg of ethylacetate extract of **3**-acid hydrolysate was chromatographed using silica gel column (10 ml wet volume) developed with stepwise gradient of hexane : ethylacetate (30 ml each of 3 : 1, 2 : 1, 1 : 1, 1 : 2). Fractions, eluted with hexane : ethylacetate (1 : 2), were collected and concentrated *in vacuo* to give 4.0 mg of 3-hydroxy-13-methyltetradecanoic acid (**9**). $[\alpha]_D^{24}$ −7.7° (*c* 0.13, $CHCl_3$). APCI-MS; *m/z* 257 $(M-H)^-$. ^1H NMR in $CDCl_3$; δ 0.78 (6H, d, J=6.6 Hz), 1.04~1.52 (19H, m), 2.31 (1H, dd, J=16.6 and 9.0 Hz 2-H_a), 2.40 (1H, dd, J=16.6 and 3.2Hz 2-H_b) and 3.96 (1H, m 3-H).

References

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NOTES

PP2A Inhibitors, Harzianic Acid and Related Compounds Produced by Fungus Strain F-1531

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Serine/threonine phosphatase type 2A (PP2A) is an intracellular protein phosphatase, which catalyzes dephosphorylation of many substrates. We have recently found that specific inhibitors of PP2A augment natural killer cells *in vivo* and inhibit tumor metastasis[1~3]. Thus, a specific inhibitor of PP2A is a candidate for a new immune activator. In the course of our searching for a novel PP2A inhibitor, we have found that the culture broth of Fungus strain F-1531 showed potent inhibitory activity against PP2A. We isolated active materials including two new compounds. These compounds were found to be active only under the chelated condition with zinc ion. In this paper, we describe the fermentation, isolation, physico-chemical properties, and biological activities of harzianic acid-related compounds.

Fungus strain F-1531 was isolated from a soil sample collected in Amagi, Shizuoka prefecture, Japan. Strain F-1531 grown on a agar slant was inoculated into 100 ml of medium containing potato starch 2%, glycerin 1%, soy bean meal 2%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_20$ 0.05% and five glass beads, and cultured at 25°C for 3 days on a rotary shaker (225 rpm). One ml of the seed culture was inoculated into 500-ml flask containing 100 ml of a culture medium containing corn starch 2%, potato starch 1%, beet sugar 1%, Pharmamedia 1%, gluten meal 1%, malt extract 0.5%, $ZnSO_4$ 0.01%, Al_2O_3 0.2%, $CaCO_3$ 0.2% (pH6.0 before sterilization) and cultured at 25°C for 4 days on a rotary shaker (225 rpm).

The fermented broth (10 liters) was filtered and the mycelia were extracted with MeOH. The mycelial extract was concentrated and combined with the broth filtrate and further extracted with BuOH. The organic layer was concentrated under reduced pressure and applied to a silica gel column prepacked with BuOAc : BuOH : MeOH : H_2O= 4 : 4 : 1 : 2. After the column was washed with the same solvents, the active materials were eluted with BuOH : MeOH : H_2O=4 : 1 : 2. Further purification was carried out by Sephadex LH-20 chromatography using MeOH as an eluent. By repeating this procedure three times, 89.7 mg of **1** was obtained as a yellow powder. Compund **1** (tentatively named **1a**) inhibited PP2A activity at IC_{50} value of 10 μg/ml without effect on other serine/threonine phosphatase type 1 (PP1). On the other hand, compound **1** (tentatively named **1b**) which was purified by reversed phase HPLC (Inertsil ODS-3, GL Science) with 80% MeOH in 20 mM KH_2PO_4 at pH 2 did not show any activity against PP2A. Thus, we examined the structural discrepancy between **1a** and **1b**. All NMR spectra including 2D NMR experiments showed that **1a** and **1b** were essentially the same to harzianic acid,[4] although the peaks of **1a** were broaden. EDS spectra of two compounds revealed the presence of Zn in **1a** and the absence of Zn in **1b**. This was further supported by the mass spectra of two compounds. In the negative mode ESI mass spectrum of **1a**, the base peak was observed at *m/z* 794 due to $[2M+Zn]^-$, while **1b** showed the deprotonated molecular ion at *m/z* 364 as the base peak. In the ESI-MS/MS spectrum of **1a**, the daughter ion at *m/z* 364 was observed from the parent ion at *m/z* 794. These results indicated that the active form was composed of **1a** and Zn as 2 : 1 complex. On the other hand, all physico-chemical properties of **1b** are the same as reported harzianic acid[4]. Thus, we concluded that **1** was active only under the chelated conditon with zinc ion.

During the purification process, two new harzianic acid family compounds were isolated. The physico-chemical properties of demethylharzianic acid (**2**) and homoharzianic acid (**3**) were shown in Table 1. The structure determination of **2** was carried out by comparing the spectral data with those of harzianic acid[4]. The molecular formula of **2** was determined to be $C_{18}H_{25}NO_6$ (MW 351) based on the

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Table 1. Physico-chemical properties of **2** and **3**.

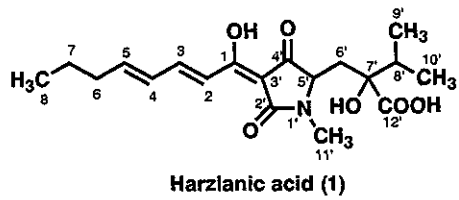
	2	**3**
Appearance	Orange Powder	Orange Powder
Molecular formula	$C_{18}H_{25}NO_6$	$C_{20}H_{29}NO_6$
ESI-MS [*m/z* (M-H)]$^-$	350 (M-H)$^-$	378 (M-H)$^-$
HRESI-MS (*m/z*)		
Calcd:	350.1577 ($C_{18}H_{24}NO_6$)	378.1917 ($C_{20}H_{28}NO_6$)
Found:	350.1586	378.1906
UV λ_{max} nm		
in MeOH:	231, 292, 350	243, 290, 344
0.01N HCl- 90% MeOH:	228, 293, 352	238, 293, 357
0.01N NaOH-90% MeOH:	246, 285, 326	249, 287, 334
Rf value on TLC [a]	0.28	0.28

[a] Silica gel 60 F_{254} (Art.5715, Merck) with BuOH-NH_4OH-H_2O-MeOH (4:1:1:0.5)

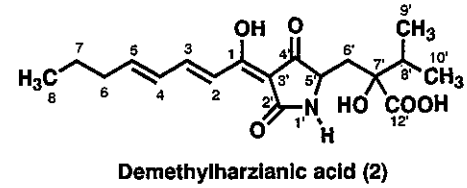
HRESI-MS and ^{13}C NMR information (Table 2). The UV spectrum of **2** was closely resemble to that of **1**. The ^{13}C NMR, DEPT and HMQC spectra of **2** revealed the presence of eighteen carbon signals consisting of three methly, three methylene, six methine and six quaternary carbons indicating the loss of one carbon and two proton atoms compared to **1**. Two side chains in **2** were identical to those of **1** based on the ^1H-^1H COSY and HMBC correlation suggesting that the structural difference between **1** and **2** should occur in five membered rings. In the ^1H NMR spectra, *N*-methyl protons (δ_H 2.94) appeared in **1** was not observed in **2** (Table 2). Any other differences were not observed in all NMR spectra. Thus, the structure of **2** was proposed as shown in Fig. 1.

The molecular formula of **3** was elucidated as $C_{20}H_{29}NO_6$ (MW 379) based on the HRESI-MS and ^{13}C NMR information. The UV spectrum of **3** also showed the similarity to **1** and **2**. The ^1H and ^{13}C NMR spectra of **1** and **3** were also similar to each other except for one additional methylene in **3**, indicating the isopropyl group of **1** was replaced by *sec*-butyl group in **3** (Table 2). This *sec*-butyl group was confirmed by cross peaks from methyl protons (H-11) to one methylene carbon (C-10), and one methine carbon in the HMBC spectrum. The remaining parts of **3** were identical with those of **1**. Thus, the structure of **3** was

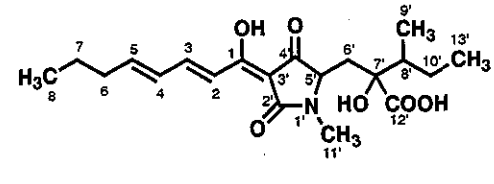
Fig. 1. Structures of harzianic acid-related compounds.



Harzianic acid (1)



Demethylharzianic acid (2)



Homoharzianic acid (3)

Table 2. ^{13}C and ^{1}H NMR assignments of **2** and **3** in chloroform-d_1.

	2		**3**	
Position	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	175.0		176.3	
2	119.1	7.14 (d, *J*=15.3Hz)	119.1	7.00 (d, *J*=15.3Hz)
3	146.9	7.51 (m)	147.5	7.55 (m)
4	129.9	6.36 (m)	129.6	6.37 (m)
5	149.5	6.36 (m)	149.9	6.37 (m)
6	35.5	2.22 (dt, *J*=6.7, 7.3Hz)	35.5	2.23 (dt, *J*=6.0, 7.3Hz)
7	21.8	1.49 (m)	21.8	1.49 (m)
8	13.7	0.94 (t, *J*=7.3Hz)	13.7	0.94 (t, *J*=7.3Hz)
2′	172.7		173.2	
3′	99.0		98.7	
4′	195.6		197.3	
5′	59.4	4.25 (dd, *J*=10.7, 2.7Hz)	64.0	3.63 (dd, *J*=10.7, 2.7Hz)
6′	38.1	2.04 (dd, *J*=12.0, 10.7Hz)	33.8	1.91 (dd, *J*=14.0, 10.7Hz)
		2.49 (dd, *J*=12.0, 2.7Hz)		2.47 (dd, *J*=14.0, 2.7Hz)
7′	77.2		80.5	
8′	36.0	1.98 (m)	42.7	1.73 (m)
9′	17.1	0.94 (d, *J*=6.7Hz)	12.3	0.97 (d, *J*=8.0Hz)
10′	16.2	1.02 (d, *J*=6.7Hz)	24.2	1.26 (m)
				1.50 (m)
11′			12.2	0.92 (t, *J*=7.3Hz)
12′	181.2		176.7	
13′			26.5	2.96 (s)

Chemical Shifts in ppm from TMS as internal standard.

^{1}H and ^{13}C NMR were measured at 400 MHz and 100 MHz, respectively.

determined as shown in Fig. 1.

These compounds weakly inhibited the growth of human prostate cancer DU-145 cells with IC_{50}s 17 (**1**), 25 (**2**), and 10 (**3**) μg/ml.

Acknowledgement

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ICM0301s, New Angiogenesis Inhibitors from *Aspergillus* sp. F-1491

I. Taxonomy, Fermentation, Isolation and Biological Activities

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In the course of screening program for inhibitors of angiogenesis, novel substances designated as ICM0301A~H (**1**~**8**) were isolated from the culture broth of *Aspergillus* sp. F-1491. ICM0301s inhibited the growth of human umbilical vein endothelial cells (HUVECs) induced by basic fibroblast growth factor (bFGF) with IC_{50} values of 2.2~9.3 μg/ml. ICM0301A (**1**) showed significant anti-angiogenic activity at lower than 10 μg/ml in the angiogenesis model using rat aorta cultured in fibrin gel. ICM0301s showed very low cytotoxicity against various tumor cells. Furthermore, ICM0301A did not show any toxic symptom in mice by intraperitoneal injection at 100 mg/kg.

Angiogenesis is the process of the formation of new blood vessels from preexisting blood vessels[1,2]. This process plays a key role in the development and wound healing. Furthermore, angiogenesis is essential for the development of solid tumor[3], metastasis of tumors cells[4] and chronic inflammation such as rheumatoid arthritis[5]. The process of angiogenesis[6,7] consists of: (i) degradation of basement membrane by MMPs; (ii) migration of blood endothelial cells (ECs); (iii) growth of ECs induced by growth factors such as bFGF and vascular endothelial growth factor (VEGF); (iv) tube formation of ECs and (v) the maturation of tube to vessel. Thus, each process of angiogenesis should be a target for development of anti-tumor and anti-inflammatory agents. In fact, TNP-470[8], which shows very strong inhibitory activity against growth of ECs, antibodies against various growth factors[9,10], anti-$\alpha V\beta 3$ integrin antibody[11], mimic peptide of RGD motif[12] contained in integrins and kinase inhibitors of VEGF receptors[13] have been developed in clinical trials.

We have screened for angiogenesis inhibitors, which exhibit inhibitory activity against the growth of HUVECs induced by bFGF, among metabolites of microorganisms. In the course of screening, ICM0301A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), G (**7**) and H (**8**) (Fig. 1) were isolated from the culture broth of *Aspergillus* sp. F-1491. In this paper, we describe the taxonomy of the producing organism, and the fermentation, isolation and biological activities of ICM0301s.

Materials and Methods

Materials

Inertsil ODS-3 columns and silica gel (Wako gel C-200) were obtained from GL Science (Tokyo, Japan) and Wako Chemical (Osaka, Japan), respectively. HUVECs and bFGF were obtained from Dainippon Pharmaceuticals (Osaka, Japan) and PEPRO TECH EC Ltd. (London, UK), respectively. Culture plate coated with collagen Type I was obtained from Sumitomo Bakelite Co. (Tokyo, Japan).

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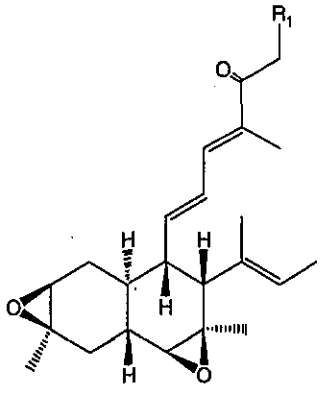
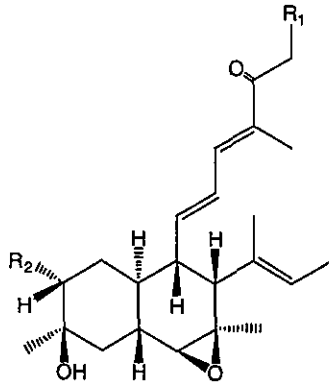
Fig. 1. Structure of ICM0301s.



ICM0301A (1) R_1=CH_3

ICM0301B (2) R_1=H



ICM0301C (3) R_1=CH_3, R_2=Cl

ICM0301D (4) R_1=H, R_2=Cl

ICM0301E (5) R_1=CH_3, R_2=H

ICM0301F (6) R_1=H, R_2=H

ICM0301G (7) R_1=H, R_2=OCH_3

ICM0301H (8) R_1=H, R_2=OH

RPMI1640, DMEM medium and HANK's balanced salt solution were obtained from Nissui Seiyaku Co. (Tokyo, Japan), and MCDB-131 medium was obtained from Kurorera Kogyo Co. (Tokyo, Japan), respectively. Bovine thrombin and fumagillin were obtained from Sigma (St. Louis, MO, USA). Bovine fibrinogen was obtained from Ito Ham (Hyogo, Japan). Lys- and gelatin-Sepharose 4B were obtained from Amershan Bioscience Co. (Piscutaway, NJ, USA).

Animals

Female ICR mice and male SD rats were obtained from Charles River Japan (Kanagawa, Japan), and were maintained under specific pathogen-free conditions at 23±1°C and 55±5% humidity.

Taxonomic Study

The producing strain, F-1491 was isolated from a soil sample collected at Kanagawa prefecture. The taxonomic studies of strain F-1491 were carried out according to the methods of PITT[14] and CARMICHAEL *et al.*[15]. The color guide of KORNERUP and WANSCHER[16] was used for determining and standardizing colors. Morphological observation of strain F-1491 was carried out using a light microscope and a scanning electron microscope.

Fermentation

The seed medium was composed of glycerin 2%, potato starch 2%, soy bean meal 2%, KH_2PO_4 0.1% and $MgSO_4 \cdot 7H_2O$ 0.005%. The seed culture was incubated at 25°C for 3 days on a rotary shaker at 225 rpm using 50 ml of medium containing 5 glass beads in a 500 ml Erlenmeyer flask. The production medium was composed of glycerin 5%, potato extract (hot water extract of 20% minced potato) 25%, malt extract 0.5%, yeast extract 0.5%, tryptone peptone 1% and Span 20 (antifoam) 0.025%, and adjusted to pH 6.5. The production culture was incubated at 25°C for 4 days on a rotary shaker at 225 rpm using 50 ml of medium in a 500 ml Erlenmeyer flask.

HPLC Analysis and Preparative HPLC

Inertsil ODS-3 columns were used for HPLC analysis (4.6×150 mm, mobile phase: 50% acetonitrile) and preparative HPLC (20×250 mm, mobile phase: 35 or 70% acetonitrile). The detection of IMC0301s was performed using ultra violet absorption at 280 nm.

Growth Inhibitory Activity against HUVECs

The inhibitory activities of ICM0301s against the growth of HUVECs were assessed as follows. HUVECs were cultured in MCDB-131 medium supplemented with 10%

FCS and 10 ng/ml of bFGF at 2×10^3 cells/100 μl in 96 wells culture plate coated with collagen Type I, and then test samples dissolved in DMSO were added to the culture. Cells were cultured for 36 hours at 37°C in 5% CO_2-air, and were further pulsed with ^{3}[H]TdR (7.4 KBq/well) for 12 hours. Proliferation of the cells was assessed by measuring incorporated radioactivity of ^{3}[H]TdR into cells using a β-ray counter.

Cytotoxicity against Tumor Cells

The cytotoxic activities of ICM0301s against human tumor cell lines including chronic myelogenous leukemia K562, non-small cell lung carcinoma H226, prostate carcinoma DLD-1 and fibrosarcoma HT1080 were assessed. These cells were cultured at 5×10^3 cells/100 μl in RPMI1640 or DMEM medium supplemented with 10% FCS for 3 days with the test samples, and proliferation of these cells was measured by the MTT method.

Anti-angiogenic Activities in Rat Aorta Organ Culture

Rat aorta organ culture was done by the methods reported by NICOSIA R. F. *et al.*[17] with some modifications. Thoracic aortas were removed from male SD rats under anesthesia using pentobarbital, and immediately transferred to a culture dish containing ice-cold serum-free HANK's balanced salt solution. The peri-aortic fibroadipose tissue was carefully removed with fine microdissecting forceps and iridectomy scissors paying special attention not to damage the aortic wall. Aortas were sectioned within small fragments (2×2 mm), and embedded in 0.5 ml of ice-cold 0.3% bovine fibrinogen (passed through gelatin- and Lysine-Sepharose) gel-MCDB131 solution on 24-wells culture plate. Clotting was obtained by adding 20 μl of a 50 NIH units/ml bovine thrombin solution to 1 ml of fibrinogen solution. The fibrin gels formed within 30 seconds at room temperature. After polymerization, 0.5 ml of MCDB 131 medium containing ε-aminocaproic acid was added to the gels, and then compound **1** or fumagillin, as a positive control, dissolved in DMSO was added to the culture. The concentration of ε-aminocaproic acid was 300 μg/ml during the first 2 days of culture followed by 50 μg/ml for the remainder of the experiment. The cultures were kept at 37°C in 5% CO_2-air. The culture medium was changed every another day. At 7 days after the start of culture, the number of tubes derived from aorta fragment was measured by light microscope observation.

Anti-microbial Activity and Toxicity in Mice

Anti-fungal activities of ICM0301s were measured by the agar dilution method. Compound **1** was dissolved in 5% DMSO-saline solution and injected to female ICR mice intraperitoneally. Body weight changes of mice were monitored for 2 weeks.

Results and Discussion

Taxonomic Studies

The fungal strain F-1491 was cultured on various media at 25 or 37°C for 7 days. The cultural characters are summarized in Table 1. For media tested the growth rates of the strain F-1491 were greatest on medium CYA. Colony surfaces on every media were flat to centrally raised, cottony to felty and white to reddish gray color. Soluble pigment was not found in the culture on every media.

Morphological characteristics (Fig. 2) of the strain were

Table 1. Cultural characteristics of strain F-1491.

Media	Diameter of Colony (mm)	Color		Surface Characteristics	Pigment or Exudate
		Surface	Reverse		
CYA*	56-58	White ~ Reddish Gray (8A~B1-2)	White ~ Reddish Gray (8A~B1-2)	Cottony to Felty	Clear Exudate No Pigment
CYA**	24-26	White ~ Reddish Gray (8A~B1-2)	White~Reddish Gray (8A~B1-2)	Cottony to Felty	None
MEA	28-29	White ~ Reddish Gray (8A~B1-2) Grayish Green*** (25C~D5-6)	White~Reddish Gray (8A~B1-2)	Cottony to Felty	None
CY20S	12-14	White ~ Reddish Gray (8A~B1-2) Grayish Green*** (25C~D5-6)	White~Reddish Gray (8A~B1-2)	Cottony to Felty	None

*: Strain F-1491 was cultured at 25 °C for 7 days.

**:Strain F-1491 was cultured at 37 °C for 7 days.

***:Strain F-1491 was cultured after several passages.