division. To further complicate analysis of events studies were frequently undertaken independently by morphological workers and biochemists.

The morphological studies revealed the patterns of cell movement allowing the investigator to analyse them in a step-by-step manner and the biochemical investigations played an important role in establishing cell membrane configurations and correlating internal cell morphology with chemical composition. However, the strength of combined morphological and biochemical analysis is apparent from the recent surge of information and as in the puzzle-review. Cells are no longer described solely in terms of their shape and all of the contributors consider the combination of morphological and biochemical information. For example, Vasiliev considers pseudopodia not only in their relation to their cytoskeletal elements, but also to their position in the culture system, and the clustering of membrane receptors. Grinnell discusses in vitro and in vivo cell-substratum interactions as a biochemical problem. With this multidisciplinary approach it has become apparent that substrata morphology and biochemistry are of even greater importance than previously anticipated. However, as more information is correlated from 3-dimensional studies with collagen lattices and Sterispon, the inherent weakness of the 2-dimensional substrate system becomes apparent. The magnitude of the 3-dimensional problem is also apparent in the difficulties which Mareel found when assessing in vitro penetration of malignant cells into normal ones by 2-dimensional methods of assessment. As a possible solution to 3-dimensional analysis Löfberg and Ebendal have suggested using embryonic tissues as models; Trinkaus and his co-workers have used such tissues to great advantage. The suggestion is an exciting one! Perhaps more workers should now return to the transparent in vivo environment of the embryo to study the movement of normal cells and their encounters with malignant ones.

SPECIALIA

The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – Ответственность за короткие сообщения несёт исключительно автор. – Solo los autores son responsables de las opiniones expresadas en estas comunicationes breves.

Microbiological transformations of β-sitosterol and stigmasterol by a soil pseudomonad[1]

S. B. Mahato and S. Banerjee

Indian Institute of Experimental Medicine, Calcutta - 700 032 (India), 18 July 1979

Summary. Fermentation of β-sitosterol by a soil pseudomonad resulted in the formation of 4-stigmasten-3-one, 4-stigmasten-3-one-6β-ol and 5-stigmasten-3β,7α-diol. In case of stigmasterol the metabolites isolated and characterized were 4,22-stigmastadien-3-one, 4,22-stigmastadien-3-one-6β-ol and 5,22-stigmastadien-3β,7α-diol.

β-Sitosterol is widely distributed in the plant kingdom. Enormous quantities of sitosterols accumulate during the isolation of stigmasterol from plant sources[2], but remain unutilized due to the absence of a suitable process for degradation of the saturated C-17 side chain. A few microbiological processes[3-5] for oxidizing and cleaving the side chain have been reported, but until now none is in use on a production basis. During our work on the isolation of microorganismus capable of cleaving the side chain of β-sitosterol we came across a soil pseudomonad capable of using β-sitosterol as a sole source of carbon. This bacterium can also convert stigmasterol in the same way as it does β-sitosterol.

The soil pseudomonad was isolated by enrichment culture on β-sitosterol. The microorganisms were found to grow on β-sitosterol and stigmasterol and produce metabolites under various aerobic conditions, but the best yields were obtained at 30 °C in mineral salt medium (substrate concentration, 500 µg/ml). Fermentation of β-sitosterol (**I**) by the bacterium yielded 3 compounds.

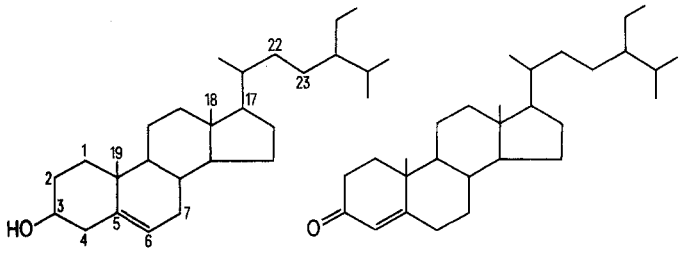
Compound 1 (yield 15%), m.p. 97–98 °C, $[\alpha]_D^{25}$ +79° (c, 1.56 in $CHCl_3$), was found to be identical with 4-stigmasten-3-one (**II**) by comparison with an authentic sample.

Compound 2 (yield 2%), $C_{29}H_{48}O_2$ (M^+ 428), m.p. 207–209 °C, $[\alpha]_D^{25}$ +18° (c, 0.56) was characterized as 4-stigmasten-3-one. 6β-ol (**III**) by comparison with an authentic specimen prepared from β-sitosterol[6].

Compound 3 (yield 2%), $C_{29}H_{50}O_2$ (M^+ 430), m.p. 210–212 °C, $[\alpha]_D^{25}$ −70.5° (c, 0.55) gave a deep blue colour with $SbCl_3$ (in $CHCl_3$) indicating it to be a 7-hydroxy derivative of β-sitosterol[7-8]. This compound was finally characterized as 5-stigmasten-3β,7α-diol (**IV**) by direct comparison with an authentic specimen[9].

Fermentation of stigmasterol (**V**) with the bacterium also yielded three metabolites.

Metabolite 1 (yield 12%), m.p. 122–124 °C, $[\alpha]_D^{25}+57°$ (c, 1.01) was identified as 4,22-stigmastadien-3-one (**VI**).
Metabolite 2 (yield 2.2%), $C_{29}H_{46}O_2$ (M^+ 426), m.p. 212–214 °C, $[\alpha]_D^{25}+14°$ (c, 0.72) was characterized as 4,22-stigmastadien-3-one-6β-ol (**VII**).
Metabolite 3 (yield 1.8%), $C_{29}H_{48}O_2$ (M^+ 428), m.p. 205–208 °C, $[\alpha]_D^{25}-62.5°$ (c, 0.25), ν_{max}^{nujol} 3400, 3300, 1050, 1020 and 870 cm^{-1}, PMR ($CDCl_3$) δ 3.85 (1 H, m, 7-*H*), 3.52 (1 H, m, 3-*H*), 5.53 (1 H, m, 5-*H*) and 5.11 (2 H, m, 22-*H* and 23-*H*) formed a dibenzoate, m.p. 165–166 °C, $[\alpha]_D^{25}-90°$ (c, 0.18) and an amorphous diacetate indicating it to be a diol. In analogy with **IV**, obtained from β-sitosterol this metabolite 3 was expected to be 5,22-stigmastadien-3β, 7α-diol (**VIII**) which is a hitherto unreported compound. This was in fact found to be so. Stigmasteryl acetate on oxidation with CrO_3-pyridine in methylene chloride[10] afforded 3β-acetoxy-5,22-stigmastadien-7-one, m.p. 181–183 °C, $[\alpha]_D^{25}-100.5°$ (c, 0.48). This keto-acetate on LAH reduction yielded 2 products. The minor product, which was assigned the α-configuration in analogy with the cholesterol series[11] was found to be identical with metabolite 3. The major product, m.p. 215–217 °C, $[\alpha]_D^{25}+32.5°$ (c, 0.35), dibenzoate m.p. 177–178 °C, $[\alpha]_D^{25}+103°$ (c, 0.38) was assigned the β-configuration. The molecular rotation differences ($M_D^{\beta}-M_D^{\alpha}$) of 407 for the diols and of 1226 for the dibenzoates are comparable with the corresponding values, viz. 378 and 1215, observed in the cholesterol series[12].



I **V** Δ^{22} **II** **VI** Δ^{22}
IV 7α–OH **VIII** Δ^{22}, 7α–OH **III** 6β–OH **VII** Δ^{22}, 6β–OH

1 The soil pseudomonad strain (BS-305) is maintained at 4 °C on nutrient-agar slants.
2 W. Charney and H.L. Herzog, Microbial Transformation of Steroids. Academic Press, New York 1967.
3 C.J. Sih und K.C. Wang, J. Am. chem. Soc. *87*, 1387 (1965).
4 M. Nagasawa, N. Watanabe, H. Hashiba, G. Tamura and K. Arima, Agric. Biol. Chem. *34*, 798 (1970).
5 W.J. Marshek, S. Kraychy and R.D. Muir, Appl. Microbiol. *23*, 72 (1972).
6 M.G. Nair and F.C. Chang, Phytochemistry *12*, 903 (1973).
7 I.M. Heilbron, T. Barr, E.G. Parry and F.S. Spring, J. chem. Soc. *16*, 1437 (1936).
8 G.A.D. Halsewood, Biochem. J. *33*, 709 (1939); *36*, 389 (1942).
9 S.C. Pakrashi, B. Achari and P.C. Majumdar, Indian J. Chem. *13*, 755 (1975).
10 W.G. Dauben, M. Lorber and D.S. Fullerton, J. org. Chem. *34*, 3587 (1969).
11 L.F. Fieser, M. Fieser and R.N. Chakravarti, J. Am. chem. Soc. *71*, 2226 (1949).
12 L.F. Fieser and M. Fieser, Steroids, p. 161. Reinhold, New York 1959.

The C-terminal sequence of human and porcine antithrombin III and its homology with human α-1-proteinase inhibitor

T. Koide, Y. Ohta and T. Ono

Department of Biochemistry, Niigata University School of Medicine, 757, Asahimachi-dori 1, Niigata 951 (Japan), 2 July 1979

Summary. The C-terminal amino acid sequences of human and of porcine antithrombin III have been determined as Gly-Arg-Val-Ala-Asn-Pro-Cys-Val-Lys and Gly-Arg-Val-Ala-Asn-Pro-Cys, respectively. These sequences are highly homologous with the C-terminal sequence of human α-1-proteinase inhibitor.

Antithrombin III (AT-III) is a plasma glycoprotein which plays an important role in the regulation of blood coagulation[1]. A deficiency of this protein leads to recurrent thromboses[2]. The inhibition of thrombin by AT-III is reported to involve a carboxylic ester formation between enzyme and inhibitor, preceded by a peptide bond cleavage at the reactive site of the inhibitor[3]. However, its exact location has not been elucidated yet. We present here data showing human and porcine AT-III contain an identical carboxy-terminal sequence which is very similar to that of human α-1-proteinase inhibitor (α-1-PI), which suggests a location for the reactive sites of the inhibitors.
Human and porcine AT-III were prepared by affinity chromatography on heparin-agarose as described elsewhere[4]. S-pyridylethyl (PE) human AT-III was digested with cyanogen bromide and the resultant peptides were fractionated by gel filtration on Sephadex G-50 (figure 1). 9 UV-absorbing peaks were obtained. Peak VII (shown by the arrow) was a mixture of 2 small peptides. It was gel-filtered on a Bio-Gel P-6 column (1.2 × 137 cm), equilibrated with 0.1 M formic acid. 2 completely separated peaks were obtained, the former of which (C-VII-1) consisted of 9 amino acids and contained no homoserine or its lactone, indicating that the peptide was derived from the C-terminal end of the protein (table). Gel filtration of the cyanogen bromide digest of S-pyridylethyl porcine AT-III on the same column of Sephadex G-50 gave 8 peaks with

Amino acid compositions of the carboxy-terminal peptides of human and porcine antithrombin III

	Human AT-III		Porcine AT-III	
	C-VII-1	Sequence	C-VII	Sequence
Asp	0.99	1	1.04	1
Pro	1.03	1	1.01	1
Gly	1.00	1	1.02	1
Ala	0.97	1	1.00	1
Val	1.96	2	0.94	1
Lys	0.92	1	0	
PECys	0.55	1	0.67	1
Arg	0.86	1	0.98	1
Total		9		7
Yield		56% (0.40 µmole)		69% (0.63 µmole)