

Tellurite Reduction in *Yersinia*

In an attempt to find further biological differences between *Yersinia malassezii* (*Pasteurella pseudotuberculosis*) and *Y. enterocolitica*, the growth of strains of the latter group on tellurite medium was observed. It has already been reported¹ that *P. pseudotuberculosis* does grow on tellurite media. Now some other strains of this group were used for this comparative study.

Ten strains of *Y. enterocolitica* and 14 strains of *P. pseudotuberculosis* (type I-V) were obtained through the courtesy of Dr. H. H. MOLLARET, Paris.

The Clauberg tellurite medium was used. Before testing, the strains of both groups were subcultured twice on 5% bovine blood agar. In the tests the inoculum was large and was made as equal as possible in both groups. The incubation was at 37°C for 48 h.

All strains of *P. pseudotuberculosis* grew to visible colonies on the tellurite medium, whereas the strains of *Y. enterocolitica* did not. Only occasional small colonies appeared in some strains of *Y. enterocolitica* during the second half of the 48 h incubation in the repeated experiments with still larger inoculums.

The growth of all strains of *P. pseudotuberculosis* on tellurite medium was abundant also in repeated experiments with smaller inoculums. The only exception was

the strain 52, type V (strain 25 of Thal) the growth of which was feeble and delayed. It will be described more extensively elsewhere.

So far, the observations seem to indicate that *Y. enterocolitica* is devoid of tellurite reductase whereas this enzyme is present in *P. pseudotuberculosis*.

Comparative studies should be extended to other organisms of the group, especially to *Y. pestis*, because the results might prove useful for the differential diagnosis of these and related bacteria².

Zusammenfassung. In Gegensatz zu *Yersinia malassezii* scheint *Y. enterocolitica* keine Tellurit-Reduktase zu besitzen.

B. BRZIN

*Institute of Microbiology, Ljubljana (Yugoslavia),
10 October 1967.*

¹ B. BRZIN, Zentbl. Bakt. Parasitkde 189, 543 (1963).

² My thanks are due to Dr. H. H. MOLLARET for giving the strains of *Pasteurella pseudotuberculosis* and *Yersinia enterocolitica* at my disposal.

COGITATIONES

Triterpènes XVI¹. Fréquence et site d'oxygénation des triterpènes naturels

Les remarques qui suivent portent sur la comparaison de 94 représentants naturels du groupe de la β -amyrine et de 73 triterpènes tétracycliques² connus au début de 1967. Les tétracycliques seront, pour notre présent propos, considérés globalement, sans distinction de sous-groupes. Les triterpènes pentacycliques des groupes de l' α -amyrine, du lupéol et a fortiori des groupes mineurs ne seront pas envisagés ici, le nombre relativement restreint de leurs représentants connus ne permettant pas encore de généralisation, quoiqu'ils semblent s'intégrer dans l'ensemble des données ci-dessous rapportées.

Il nous a paru intéressant de calculer le pourcentage des triterpènes naturels oxygénés en chacune des positions du squelette et de comparer les résultats obtenus dans les 2 séries en faisant correspondre les atomes de carbone biogénétiquement équivalents.

Tous les triterpènes β -amyréniques et 92% des tétracycliques³ sont oxygénés en C-3. Cette prédominance bien connue est en accord avec la structure du précurseur commun actuellement admis, le 2, 3-oxydosqualène⁴.

Les 2 séries diffèrent principalement en ce que 21% des β -amyréniques sont oxygénés en C-23, fait dont on ne retrouve aucunement l'équivalent chez les triterpènes tétracycliques où, dans le groupe lanosténique, l'oxygénation des méthyles portés par C-4 semblerait mener rapidement, par attaques ultérieures, à la série stéroïdique⁵. On constate, d'autre part, que le carbone C-20 est oxydé chez 22% des triterpènes tétracycliques, alors que son homologue C-17, quaternaire en série β -amyrénique, ne pourrait l'être.

Venant après le carbone C-3, la position la plus fréquemment oxygénée en série β -amyrénique est C-28 (71%), dont les voisins C-16 et C-22 sont oxydés à raison de 34% et 24%, respectivement. Il en va de même pour leurs équivalents biogénétiques C-21, C-16 et C-22 en série tétracyclique, oxygénés à raison de 23%, 14% et 7%. Il nous faut constater, en outre, que les fréquences relatives d'oxygénation de ces positions sont très semblables dans les 2 groupes (environ 10/5/3,5 en série β -amyrénique et 10/6/3 en série tétracyclique) bien que leur disposition – et partant leur accessibilité – soit nettement différente.

Une rationalisation possible de ces observations serait d'admettre que l'oxygénation privilégiée du C-28 (et, peut-être, de C-16 et C-22) des triterpènes β -amyréniques

¹ Partie XV. B. TURSCH, D. DALOZE, C. BARTHOLOMÉ et G. CHIURDOGLU, Bull. Soc. chim. Belg., à paraître.

² A l'exception du groupe de la limonine et de ses analogues hautement oxygénés.

³ La numérotation adoptée ici est celle de P. BOITEAU, B. PASICH et R. RATSIMAMANGA, *Les Triterpénoides en Physiologie végétale et animale* (Gauthiers-Villars Paris, 1964).

⁴ E. J. COREY, W. E. RUSSEY et P. R. ORTIZ DE MONTELLANO, J. Am. chem. Soc. 88, 4750 (1967) et E. E. VAN TAMMELN, E. E. WILLETT, R. B. CLAYTON et K. E. LORD, J. Am. chem. Soc. 88, 4752 (1967).

⁵ Pour une revue récente voir R. B. CLAYTON, Q. Rev. chem. Soc. 19, 168 et 201 (1965).

se produirait, en fait, au niveau d'un précurseur tétracyclique (du type dammarenediol, par exemple), c'est-à-dire avant la formation du cycle E.⁶

⁶ C. DJERASSI (*Cactus Triterpenes*, Festschrift ARTHUR STOLL 1957, p. 330) avait déjà considéré l'hypothèse d'une relation intime entre l'oxygénation et le processus de formation du squelette.

Summary. Comparison of the known tetracyclic and β -amyrenic triterpenes shows that natural oxygenation of the skeleton occurs preferentially at certain carbon atoms that are biogenetically equivalent in the 2 series.

B. TURSCH et J. LECLERCQ

Service de Chimie Organique E.P., Université Libre de Bruxelles (Belgique), 10 novembre 1967.

STUDIORUM PROGRESSUS

On the Relationship Between Glucagon and Secretin

In recent years considerable effort has been devoted to an analysis of the observed alterations of amino acid residues in specific, biologically active hormones, enzymes and proteins as a guide to the zoological cognation and the phylogenetic distance between 2 species¹⁻⁴. Such techniques are dependent upon comparisons between homologous proteins and have been greatly aided by studies with cytochrome *c*⁵⁻⁸, hemoglobin⁸⁻¹¹ and various proteolytic enzymes¹². A similar approach is made at this time for the important polypeptides glucagon and secretin.

Glucagon was originally isolated as a byproduct of insulin research¹³. The marked hyperglycemic action of the material led to the name, which means 'mobilizer of sugar'¹⁴. Later, sufficient amounts of crystalline glucagon were obtained from the α -cells of the pancreas¹⁵ and were used to determine both the composition and the amino acid sequence of the hormone¹⁶. Several analytical methods for the hormone are available and these serve as useful adjuncts in the treatment of glycogen storage diseases by glucagon^{17,18}. The chemical and medical applications of glucagon have been reviewed recently^{19,20}.

Secretin possesses a shorter history, but due to the marked advancement in the use of physical instrumentation, the identification²¹, isolation²² and structure determination²³ of the material proceeded at a rapid pace. This new hormone is found in the cell walls of the duodenum and acts by stimulating the pancreas to release sodium bicarbonate, hence the name. Within the past year the complete structure of the compound was announced²⁴ and was followed in turn by a complete synthesis²⁵. Contemporary developments in this field have been summarized, too²⁶.

The nonacosapeptide sequence of glucagon (porcine) bears a remarkable resemblance to the heptacosapeptide sequence of secretin (porcine) (see Scheme 1). Indeed, there are 15 invariant and positionally identical amino acids in both compounds: A₁, A₂, A₄, A₅, A₆, A₇, A₈, A₁₁, A₁₅, A₁₆, A₁₈, A₂₀, A₂₄ and A₂₆. Application of the 'index of similarity'²⁷ to secretin and the first 27 residues of glucagon gives a sum of $14/27$, or 0.52. The result definitely suggests a common origin for the 2 compounds, probably stemming from the process of genetic duplication. A structural parallelism was detected earlier in a computer comparison of the residues in glucagon (bovine?) and secretin (porcine)²⁸ and it was noted that the pancreas and duodenum have a common embryonic background, a fact of some evolutionary significance²⁹.

¹ E. ZUCKERKANDL and L. PAULING, *Evolving Genes and Proteins* (Academic Press, New York 1965), p. 97.

² R. ARCHER, *Angew. Chem. Int. Ed.* 5, 798 (1966).

³ M. FLORKIN, *A Molecular Approach to Phylogeny* (Elsevier, Amsterdam 1966).

⁴ T. H. JUKES, *Molecules and Evolution* (Columbia University Press, New York 1966).

⁵ E. MARGOLIAH and E. L. SMITH, *Evolving Genes and Proteins* (Academic Press, New York 1965), p. 221.

⁶ E. MARGOLIAH and A. SCHEJTER, *Adv. Protein Chem.* 21, 113 (1966).

⁷ C. R. CANTOR and T. J. JUKES, *Biochem. biophys. Res. Commun.* 23, 319 (1966).

⁸ C. R. CANTOR and T. J. JUKES, *Proc. natn. Acad. Sci., U.S.* 56, 177 (1966).

⁹ J. BUETTNER-JANUSCH and R. L. HILL, *Evolving Genes and Proteins* (Academic Press, New York 1965), p. 167.

¹⁰ E. ZUCKERKANDL, *Protides of the Biological Fluids* (Elsevier, Amsterdam 1965), p. 102.

¹¹ W. M. FITCH, *J. molec. Biol.* 16, 17 (1966).

¹² H. NEURATH, K. A. WALSH and W. P. WINTER, *Science* 158, 1638 (1967).

¹³ C. B. F. GIBBS, E. W. ROOT JR. and J. R. MURLIN, *Q. Jl exp. Physiol.* 13, 128 (1923).

¹⁴ C. P. KIMBALL and J. R. MURLIN, *J. biol. Chem.* 58, 337 (1923).

¹⁵ A. STAUB, L. E. SINN and O. K. BEHRENS, *J. biol. Chem.* 214, 619 (1955).

¹⁶ W. W. BROMMER, L. G. SINN and O. K. BEHRENS, *J. Am. chem. Soc.* 79, 2807 (1957).

¹⁷ W. W. BROMMER and O. K. BEHRENS, *Methods in Hormone Research* (Academic Press, New York 1962), p. 459.

¹⁸ E. W. PROBST and R. W. COLWELL, *Biochemistry* 5, 1209 (1966).

¹⁹ O. K. BEHRENS and W. W. BROMER, *Vitamins Horm.* 16, 263 (1958).

²⁰ P. P. FOA and E. GALASINO, *Glucagon: Chemistry and Function in Health and Disease* (Charles C. Thomas, Springfield 1962).

²¹ J. E. JORPES and V. MUTT, *Ann. intern. Med.* 55, 395 (1961).

²² J. E. JORPES and V. MUTT, *Acta chem. scand.*, 15, 1790 (1961).

²³ V. MUTT, S. MAGNUSSON, J. E. JORPES and E. DAHL, *Biochemistry* 4, 2358 (1965).

²⁴ V. MUTT and J. E. JORPES, *Fourth Int. Symp. Chem. Nat. Products*, Stockholm (1966).

²⁵ M. BODANSZKY and N. J. WILLIAMS, *J. Am. chem. Soc.* 89, 685 (1967).

²⁶ V. MUTT and J. E. JORPES, *Recent Prog. Horm. Res.* 23, 483 (1967).

²⁷ A. VEGOTSKY and S. W. FOW, *Comparative Biochemistry* (Academic Press, New York 1962), vol. 4, p. 185.

²⁸ R. V. ECK and M. O. DAYHOFF, *Atlas of Protein Sequence and Structure 1966* (National Biomedical Research Foundation, Silver Spring 1966), pp. 89, 108, 109, 195.

²⁹ B. M. PATTEN, *Human Embryology* (The Blakiston Company, New York 1953), p. 479.