Als Beispiel mögen die Befunde von MUNOZ[109] dienen. Er immunisierte 18 Kaninchen in üblicher Weise mit kristallisiertem Serum-Albumin (Rind). Die Prüfung der Antisera gegen das zur Injektion verwendete Albuminpräparat ergab nach der Technik von OUDIN im Agar-Gel: 1 Tier ohne Präzipitatlinie, 12 Tiere mit 1 Linie, 2 Tiere mit 2 Linien und schliesslich 3 Tiere mit 3 Linien. Solche wesentlichen Unterschiede können mit Immunoelektrophorese gut sichtbar gemacht werden[110]. Auf Abbildung 7 finden sich Präzipitatlinien, wo als Antigen jeweils normales Serum, human, im Agar-Gel aufgetrennt war mit Antisera, die in Maulesel (a), Pferd (b_1, b_2), Kaninchen (c_1, c_2, c_3), Huhn (d) und Ente (e) gegen Serum human gebildet waren, reagierten.

Der Vergleich von b_1 mit b_2 oder von c_1, c_2 mit c_3 zeigt eindrücklich die von Tier zu Tier abweichende Linienbildung. Es mag damit zusammenhängen, dass in solchen Fällen im Tierorganismus nicht nur Antikörper gegen ein einzelnes heterologes Protein gebildet werden soll, sondern gleich gegen ein ganzes Spektrum der verschiedensten Fraktionen und Unterfraktionen. Dadurch werden offenbar an den Stellen der Proteingenese Verdrängungen und Überschneidungen unvermeidlich. Es ist ebensowohl möglich, dass der Tierorganismus gegen die eine Proteinkomponente nur ungenügend Antikörper bildet wie eine extra starke Bildung begünstigt. In beiden Fällen kann man nur über passend gewählte Konzentration bzw. Verdünnung des Antiserums zu jener Gleichgewichtskonzentration gelangen, welche eine analytisch optimale serologische Reaktion gewährleistet. Auf diese Weise muss in jedem Falle ein neues Immunserum gewissenhaft ausgetestet werden. Eine gewisse Standardisierung der Resultate könnte erreicht werden, wenn die Testantigene als Bezugssubstanzen ausgetauscht würden. Diese Unsicherheit durch den «Tierfaktor» hat zur Folge, dass *Bestrebungen zur quantitativen Auswertung der Reaktion zurücktreten vor der Sicherstellung ihrer qualitativen Aussage.*

Nachdem die Immunoelektrophorese als Methode nur wenige Jahre besteht, darf erwartet werden, dass ihre Technik im Sinne einer Vereinfachung noch verbessert wird. Dessenungeachtet ist sie schon in ihren heutigen Ausführungsformen eine ausserordentlich wertvolle Methode zur serologischen Charakterisierung der verschiedensten Proteine und Proteide.

Summary

An account is given of Immuno-electrophoresis, as it originated from a combination of electrophoresis in agar-jelly and the various experiences with antigen/antibody reactions in gels. The theoretical background is detailed as well as different techniques. The increased sensitivity has gained us new insight into the composition of body fluids. Finally the limitations are discussed as they are proper to all reactions which have an immuno-chemical basis.

[109] J. MUNOZ aus: *Serolog. Appr. to studies of protein structure and metab.* (Rutgers Univ. Press 1954), S. 55.

[110] P. GRABAR, technische Mithilfe von Mlle J. COURCON (unveröffentlichte Ergebnisse).

Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

Les auteurs sont seuls responsables des opinions exprimées dans ces communications. – Für die kurzen Mitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed by their correspondents.

Antibiotics X-948 and X-1008

Two recent publications on Echinomycin[1] prompt us to publish the results of our investigation of two related antibiotics, X-948 and X-1008, of which the former is probably identical with Echinomycin. It yields the same degradation products (ammonia, quinoxaline-2-carboxylic acid, N-methyl-L-valine, L-alanine and D-serine) and resembles Echinomycin also in its physical properties[2].

Antibiotic X-948 was isolated from broth filtrates and cells of an apparently new Streptomyces species, our number X-948. This culture is possibly the same as *Streptomyces echinatus*[1], but there are at least two differences in the bacteriological characterization: *S. echinatus* does not grow on sodium citrate or liquefy gelatin, whereas *Streptomyces sp.* X-948 does both.

Antibiotic X-948 was also isolated from broths of *Streptomyces n. sp.* X-537 which produces a second crystalline antibiotic, designated as X-537A and described in 1951[3]. This streptomycete clearly differs from the

[1] R. CORBAZ, L. ETTLINGER, E. GÄUMANN, W. KELLER-SCHIERLEIN, F. KRADOLFER, L. NEIPP, V. PRELOG, P. REUSSER, and H. ZAHNER, Helv. chim. Acta *40*, 199 (1957). – W. KELLER-SCHIERLEIN and V. PRELOG, Helv. chim. Acta *40*, 205 (1957).

[2] Prof. V. PRELOG, to whom a sample of X-948 was sent, kindly compared the two antibiotics. He reported (personal communication) that they are very probably identical. He found that Echinomycin melted 8° lower than X-948, but that the mixed melting point showed no depression. The infrared absorption spectre were practically identical, with very small differences in the height of 3 weaker bands in the 8–8·5 μ region. Paper chromatography using the Bush C-system showed no differences.

[3] J. BERGER, A. I. RACHLIN, W. E. SCOTT, L. H. STERNBACH, and M. W. GOLDBERG, J. Amer. chem. Soc. *73*, 5295 (1951).

two previous organisms in its lack of dark pigment production on many media, and its fermentation reactions towards such substrates as ribose, sucrose, raffinose and sodium nitrite.

A third *Streptomyces n. sp.* X-1008, isolated in our laboratories, produces a crystalline antibiotic X-1008 that appears to be closely related to, but is definitely different from antibiotic X-948 or Echinomycin. This streptomycete differs from *Streptomyces sp.* X-948 in its growth reactions toward arabinose, xylose, cellobiose, lactose, malate and tryptophane, but its pigment production is generally parallel on media where both of the cultures grow. Qualitatively, antibiotics X-948 and X-1008 have similar antibacterial spectra; there are, however, some quantitative differences, as well as only partial crossover with bacteria made resistant to each of the two substances.

Three additional unidentified streptomycetes (X-3565, X-3593 and X-3600) have been found to produce substances with antibacterial spectra very similar to that of antibiotic X-948[4]. From one of these (culture X-3565) an antibiotic was isolated, which was crystallized from the same solvent mixture as used for antibiotic X-948. The crystals gave no melting point depression with antibiotic X-948, and had the same antibacterial activity.

On a medium containing 2% soybean flour, 2% brown sugar, 0·5% cornsteep liquor concentrate and 0·1% K_2HPO_4, broth potencies with *Streptomyces sp.* X-948 in aerated 200 gallon tank fermentations have attained values equivalent to 1 g of antibiotic/l; yields of isolated crystalline antibiotic X-948 have reached 0·2 g/l. Its activity against our test organism in a cylinder plate assay is 1500 units per mg, where 1 unit per ml is the concentration required to produce a 20 mm zone of inhibition on agar plates seeded with E bacillus. The activity of antibiotic X-1008 against E bacillus is 1000 units per mg.

Antibiotic X-948 was found by SCHNITZER[5] to have an appreciable trypanocidal effect in mice. It was, however, very toxic. Mr. B. TABENKIN and Dr. J. M. COOPERMAN of our laboratories found that crystalline antibiotic X-948 is markedly toxic when fed at a dietary concentration of 0·01% to young chicks.

For the large scale preparation antibiotic X-948 was extracted with butyl acetate from cells, which were separated by filtration from the acidified (pH 4–5) fermentation broth of *Streptomyces* X-948. The extract was concentrated *in vacuo* and diluted with petroleum ether. The precipitate was dissolved in chloroform and treated with charcoal. The solution was then diluted with methanol and the chloroform removed by distillation at atmospheric pressure. Antibiotic X-948 crystallizes under these conditions in colorless prisms, melting at 236–238° (dec.) corr.;

$$[\alpha]_D^{28} = -308° \pm 5° \ (c = 1{\cdot}2\% \text{ in } CHCl_3).$$

The UV. absorption spectrum is practically identical with that published for Echinomycin[1]. The IR. spectrum is very similar[2]. The average of many analyses of antibiotic X-948 was: C, 55·19; H, 5·94; N, 15·22; O, 17·51; S, 5·54. Calculated for $C_{29}H_{39}O_7N_7S$: C, 55·31; H, 6·24; N, 15·57; O, 17·79; S, 5·09[6].

On refluxing antibiotic X-948 with 0·4 *N* barium hydroxide, 1 mole of ammonia was evolved and the barium salt of quinoxaline-2-carboxylic acid (about 1 mole) was precipitated. Quinoxaline-2-carboxylic acid (m.p. 209–211°) was liberated with acid and identified by analysis (calculated for $C_9H_6ON_2$: C, 62·01; H, 3·48, found: C, 62·09; H, 3·81), UV. spectrum (practically identical with that of X-948), and conversion into the methyl ester, melting at 114° (calculated for $C_{10}H_8O_2N_2$: C, 63·85; H, 4·25; N, 14·91, found: C, 64·22; H, 4·39; N, 14·86). Neither the acid nor the ester gave a melting point depression with synthetic preparations. The barium hydroxide solution was freed from barium ions and concentrated *in vacuo*. The residue was dissolved in methanol and crude N-methyl-L-valine precipitated with isopropanol. Recrystallized from a mixture of water, methanol and acetone, this amino acid forms flat needles, subliming at 200° without melting (calculated for $C_6H_{13}O_2N$: C, 54·94; H, 9·99; N, 10·68, found: C, 55·13; H, 9·89; N, 10·49);

$$[\alpha]_D^{25} = +15{\cdot}1 \ (c = 3\%, H_2O); \ [\alpha]_D^{25} = +31{\cdot}0 \ (c = 1{\cdot}4, 5N \text{ HCl})^7.$$

Antibiotic X-948 was also hydrolyzed by refluxing for 5 h with 5·5 *N* HCl. The mixture was concentrated, diluted with water and extracted with ether[8]. The aqueous solution was filtered and reacted with 2,4-dinitrochlorobenzene at 80°. The reaction products were extracted with ether and the extract was concentrated *in vacuo*. The residual orange oil (1 part) was dissolved in ether, diluted with the same volume of petroleum ether, and adsorbed on a distribution column, prepared by suspending a mixture of 30 parts of silica gel (100–200 mesh), 10 parts Hyflo Super-cel, 10 parts of 1 molar pH 8 potassium phosphate buffer in ether-petroleum ether (50:50). The column was eluted with mixtures of ether and petroleum ether (1:1 and 9:1), saturated with the above buffer. The first fraction yielded 2,4-dinitrophenyl-N-methyl-L-valine, melting at 181–184°;

$$[\alpha]_D^{27} = +495° \pm 10° \ (c = 0{\cdot}65\% \text{ in chloroform}).$$

Calculated for $C_{12}H_{15}O_6N_3$: C, 48·48; H, 5·09; N, 14·14, found: C, 48·92; H, 5·45; N, 14·39. A mixed melting point with a preparation obtained by PLATTNER and NAGER from Enniatin B gave no depression[9]. The second fraction yielded about 0·75 moles of 2,4-dinitrophenyl-L-alanine, melting at 176–177°;

$$[\alpha]_D^{26} = +10{\cdot}5° \pm 2° \ (c = 2 \text{ in absolute ethanol}^{10}).$$

[4] *Streptomyces sp.* X-948 was isolated from a soil sample obtained in Shreveport, La., U.S.A., X-537 from a soil sample of Hyde Park, Mass., U.S.A., and X-3565, X-3593, and X-3600 from three different soil samples of Colombia, S.A.

[5] R. J. SCHNITZER, Ann. N. Y. Acad. Sci. *55*, 1090 (1952).

[6] Suggested for Echinomycin: $C_{29}H_{37}O_7N_7S$. Other formulas, such as $C_{25}H_{32}O_6N_6S$, would also fit our analytical results.

[7] PL. A. PLATTNER and U. NAGER, Helv. chim. Acta *31*, 2192 (1948), report + 17·5° (H_2O) and + 30·9° (5*N* HCl).

[8] Quinoxaline-2-carboxylic acid was never found in such hydrolysates. Since quinoxaline-2-carboxylic acid is stable under our acid hydrolysis conditions, we are inclined to believe that its formation by the degradation of antibiotic X-948 with alkali is not due to a simple hydrolysis of an ester or amide linkage, in spite of the great similarity of the UV. absorption spectra of both compounds.

[9] PL. A. PLATTNER and U. NAGER, Helv. chim. Acta *31*, 665 (1948), report m.p. 181·5–182°; $[\alpha]_D^{19} = +482{\cdot}5°$ ($c = 0{\cdot}716\%$ in chloroform). We are indebted to Prof. PLATTNER for a sample of their 2,4-dinitrophenyl-N-methyl-L-valine.

[10] It was found that the $[\alpha]_D$ of 2,4-dinitrophenyl-L-alanine in ethanol varied considerably, depending on the amount of water present: + 18·0° ± 1° in 95% ethanol, and + 21·0° ± 1·5° in 80%

Calculated for $C_9H_9O_6N_3$: C, 42·36; H, 3·56, found: C, 42·44; H, 3·46. The sample was found to be identical with a 2,4-dinitrophenyl derivative prepared from commercial L-alanine. A third fraction was obtained after extrusion of the column. The upper part of the column (yellow) was extracted with methanol. The methanol solution was concentrated *in vacuo* to a small volume. Yellow crystals of a potassium salt precipitated. After acidification in aqueous solution, 2,4-dinitrophenyl-D-serine (m.p. 177–178°) was obtained (yield 0·58 moles);

$$[\alpha]_D^{26} = -4{\cdot}3° \pm 0{\cdot}6° \ (c = 1{\cdot}5\% \text{ in absolute ethanol}^{10}).$$

Calculated for $C_9H_9O_7N_3$: C, 39·87; H, 3·35, equivalent weight 283, found: C, 40·02; H, 3·40, equivalent weight by potentiometric titration, 283, 286. The sample was compared with the 2,4-dinitrophenyl derivative prepared from commercial L-serine. The melting point was the same, the rotation opposite[10]. The racemate prepared from equal amounts of the 2,4-dinitrophenyl derivative of L-serine and our D-serine derivative was in every respect identical with the 2,4-dinitrophenyl derivative prepared from commercial racemic serine[11].

Antibiotic X-1008 was isolated from a submerged culture of *Streptomyces sp.* X-1008 by extracting the whole broth with butanol. The extract was concentrated *in vacuo* and the antibiotic precipitated with petroleum ether. It was successively extracted into methylene chloride and methanol, and then crystallized from a mixture of ethanol and acetonitrile. Antibiotic X-1008 forms cube-like crystals, melting with decomposition at 209–216° (corr.);

$$[\alpha]_D^{27} = -282° \ (c = 1\%, \text{ chloroform}).$$

It gives a melting point depression when mixed with antibiotic X-948. Its UV. absorption spectrum is practically identical with that of antibiotic X-948 and shows the presence of the quinoxaline residue. Barium hydroxide hydrolysis gives quinoxaline-2-carboxylic acid. The IR. spectrum is similar, but differs in details from that of antibiotic X-948. The analyses point to the formula $C_{29}H_{38}O_7N_6S$ (calculated: C, 56·66; H, 6·23; N, 13·67; S, 5·22, found: C, 56·40; H, 6·52; N, 13·69; S, 5·08). These results show that antibiotic X-1008 is structurally related to antibiotic X-948. However, there are only 6 nitrogens in its molecule, as against 7 in antibiotic X-948, indicating that the side chain attached to the quinoxaline residue is of a different nature.

Antibiotic X-1008 is, like antibiotic X-948, a highly toxic compound. It is, however, devoid of trypanocidal activity in mice, when given at the tolerated dose[12].

We are indebted to Dr. AL. STEYERMARK and his staff for the microanalyses and to Dr. A. MOTCHANE and his staff for the spectroscopic data.

J. BERGER, E. R. LA SALA, W. E. SCOTT, B. R. MELTSNER, L. H. STERNBACH, S. KAISER, S. TEITEL, E. MACH, and M. W. GOLDBERG

Research Laboratories, Hoffmann-La Roche, Inc. Nutley, New Jersey, USA., June 24, 1957.

ethanol. The 2,4-dinitrophenyl derivatives of D- and L-serine showed a similar behavior: $-4{\cdot}3° \pm 0{\cdot}5°$ and $+4{\cdot}4° \pm 0{\cdot}3$, respectively, in 100% ethanol, $-12{\cdot}1° \pm 1{\cdot}5°$ and $+13{\cdot}0° \pm 2°$, respectively, in 95% ethanol, and $-16{\cdot}0° \pm 1°$ for the D-form in 90% ethanol.

[11] R. R. PORTER and F. SANGER, Biochem. J. *42*, 287, (1948).

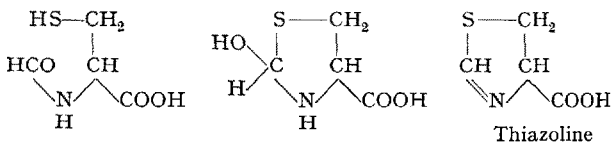
[12] Personal communication by Dr. R. J. SCHNITZER of our Chemotherapy Laboratories.

Zusammenfassung

Es werden zwei neue, chemisch verwandte Streptomyces Antibiotika beschrieben, X-948 ($C_{29}H_{39}O_7N_7S$?) und X-1008 ($C_{29}H_{38}O_7N_6S$?). X-948 ist wahrscheinlich identisch mit Echinomycin. Es hat sehr ähnliche physikalische Eigenschaften und gibt die gleichen Abbauprodukte (Ammoniak, 2-Chinoxalincarbonsäure, N-Methyl-L-valin, L-Alanin und D-Serin).

Thiazoline Carboxylic Acid from Formylcysteine

CALVIN[1] discovered that glutathione (GSH) dissolved in 12 *N* HCl gives the spectrum of a thiazoline derivative with a maximum at 268·5 mμ. By spectrometric evidence a thiazoline derivative was presumed to be produced in the course of the metabolism of thiazolidine carboxylic acid by rat liver preparations[2]. It seemed of interest to assay whether the treatment of N-formylcysteine (FC) with strong acid is followed by the ring closure to a thiazoline derivative in a manner similar to the behaviour of GSH. The expected reaction should follow the steps indicated below:



FC was prepared starting from diformylcystine[3] by the same method used by PIRIE and HELE for the preparation of acetylcysteine[4]. It was dissolved in HCl of increasing normality and the O.D. at 268·5 mμ was registered against the same solvent in function of time.

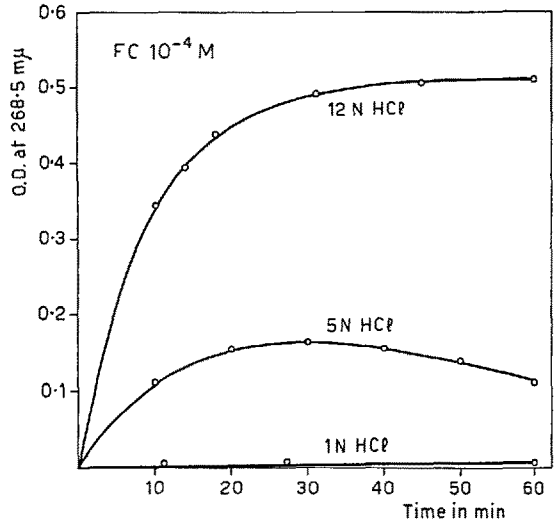


Fig. 1. — Optical density of formylcysteine dissolved in HCl of different strengths at 268·5 mμ, in function of time.

[1] M. CALVIN, *Symposium on Glutathione* (Academic Press, New York 1954).

[2] D. CAVALLINI, C. DE MARCO, B. MONDOVÌ, and F. TRASARTI, Biochim. biophys. Acta *22*, 558 (1956).

[3] V. DU VIGNEAUD, R. DORFMANN, and H. S. LORING, J. biol Chem. *98*, 577 (1932).

[4] N. W. PIRIE and T. S. HELE, Biochem. J. *27*, 1716 (1933).