de masse, ce qui correspond à la perte d'une molécule d'acide acétique et d'une molécule d'oxyde de carbone[3].

Son spectre de RMN diffère essentiellement de celui du tétraacétate IV b par l'absence du signal dû au proton H_9 et par la disparition d'un singulet de groupement acétoxy et par la présence d'un doublet à 357 Hz attribuable à un proton vinylique.

Ces modifications suggèrent que le produit de thermolyse possède trois fonctions acétoxy et une double liaison trisubstituée. Le déplacement paramagnétique du signal dû au méthyle en C-1 (145 Hz) ainsi que l'absorption UV de ce composé ($\lambda_{max} = 254$ nm; $\varepsilon = 10.120$) indique que le double liaison est conjuguée au noyau aromatique et par conséquent en position 9–12.

L'ensemble de ces résultats conduit à attribuer au produit de dégradation pyrolytique la formule VII. La formation de ce composé que nous appelons triacétyl-7, 15, 32 *bis* nor-11, 16 glaucanol, peut s'expliquer par le mécanisme schématisé ci-dessous:

Signalons que plusieurs auteurs[4-7] ont déjà rapporté la décomposition thermique de certaines α-acétoxy-cyclohexanones plus ou moins substituées. Certains des produits obtenus sont des dérivés cyclo-pentèniques qui résulteraient de la perte d'oxyde de carbone avec contraction de cycle.

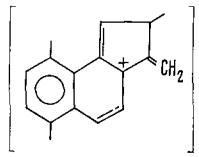
Plus récemment, CARLSON et BATEMAN[8] ont pu préparer le cyclononène par thermolyse de l'acétoxy-1 cyclo décanone. Par ailleurs, COOKSON et coll.[9] ont rapporté l'obtention du stilbène par décomposition thermique de la benzyl α-acétoxy benzyl cétone (C_6H_5–CH_2–CO–CH(OAc)–C_6H_5)[10].

Summary. The pyrolysis of 7, 12, 15, 32-tetraacetyl 16-nor glaucanol IV b (a degradation product of glaucarubin) leads to the olefin 7, 15, 32-triacetyl 11, 16-*bis* nor glaucanol VII which has a contracted ring C. A mechanism is proposed for the formation of compound VII.

J. ZYLBER

Institut de Chimie des Substances Naturelles C.N.R.S., 91 Gif-sur-Yvette (France), 18 novembre 1968.

[3] Il est intéressant de signaler que les spectres de masse du composé tétraacétylé IV b et du produit de pyrolyse VII présentent tous les deux un pic important à m/e 221. La mesure de masse de cet ion indique qu'il a la composition $C_{17}H_{17}$ (Calc. pour $C_{17}H_{17}$: 221, 1330; Tr.: 221, 1336). Nous lui attribuons la formule suivante (ou une structure mésomère).



L'obtention de cet ion à partir du composé IV b suggère qu'une contraction du cycle C analogue à celle observée au cours de la pyrolyse a lieu également lors de la mesure du spectre.

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[10] Nous remercions vivement Mme J. POLONSKY pour l'intérêt témoigné à ce travail. – Nous adressons nos remerciements à B. C. DAS et P. VARENNE pour les spectres de masse et les mesures de masse à haute résolution et à Mme L. ALAIS pour les spectres de RMN.

New Carotenoids from *Streptomyces mediolani* n. sp.

Despite the growing knowledge of the physiology of actinomycetes, the accumulation of carotenoids in microorganisms belonging to this group has so far never been clearly established. In the present study a new species of Streptomyces, named *S. mediolani*, has been found to produce up to 3% of dry weight of a mixture of carotenoid compounds, and a peptide antibiotic, when grown in shake flasks on a suitable medium. One compound has been identified as isorenieratene[1] (synonym leprotene[2]). Two other pigments were phenolic carotenoids and their structure has been determined as 3-hydroxyisorenieratene and 3, 3′-dihydroxyisorenieratene respectively. 3-Hydroxy isorenieratene shows the interesting property of improving the colour of egg yolk when administered as a feed additive to the laying hen[3].

Microorganism. Streptomyces strain 2215/74 F.I. has been isolated from a soil sample in our laboratory. Its main features are as follows: sporophores are long and straight; from these, through monopodial branching, the spore-bearing hyphae originate as long, straight to slightly flexuous filaments. Spores are cylindrical in shape, and devoid of any ornamentation. Growth is always very good on the most common agar media; the substrate mycelium grows as a compact, smooth patina, showing a yolk-yellow to orange-yellow colour. The aerial mycelium is very abundant showing a velvety to dusty aspect; its colour varies from yellow-vanilla to yellow-rose or yellow-beige, according to the different substrates on which it is grown.

It has been possible to conclude, from the examination of all the subgeneric taxa of the genus Streptomyces known in the literature, that our culture belongs to a new species, to which the name *S. mediolani* has been given.

Fermentation. The spore suspension prepared from a culture of *S. mediolani* grown on a solid medium containing yeast extract, glucose, and inorganic salts, was used to inoculate 300 ml Erlenmeyer flasks each containing 60 ml of a liquid medium having dextrin, casein, corn-steep liquor, calcium carbonate and other inorganic salts as ingredients. After 27 h of incubation at 28 °C on a rotary shaker, aliquots of the culture were transferred to 300 ml Erlenmeyer flasks each containing 30 ml of the following production medium: soluble starch, 37.5 g; morsuit (a maltose rich syrup manifactured by F.R.A.G.D.,

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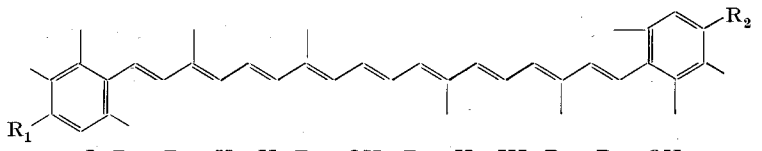

I: $R_1 = R_2 = H$; II: $R_1 = OH$, $R_2 = H$; III: $R_1 = R_2 = OH$

Italy), 33 g; soybean meal 40 g; NaCl, 1.25 g; KH_2PO_4, 3 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCO_3$, 3.5 g; $(NH_4)_2SO_4$, 1.5 g; $ZnSO_4 \cdot 7H_2O$, 0.005 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; $CuSO_4 \cdot 5H_2O$, 0.001 g; tap water up to 1000 ml. The flasks were incubated for 8 days on a rotary shaker at 29 °C.

Chemical studies. The extracts prepared from the mycelium of *S. mediolani* with the aid of different organic solvents contained 3 main carotenoids, designated A, B and C, together with other minor pigments. Compound A and B were epiphasic, while pigment C precipitated at the interfacies in the biphasic system petroleum ether (b.p. 40–60°)-90% aqueous methanol. Separation of the epiphasic pigments by chromatography on aluminum oxide, using petroleum ether containing increasing amounts of acetone as developing agent, allowed isolation of pigment A, $C_{40}H_{48}$, red crystals m.p. 200–201°, λ_{max} (petroleum ether) 278, 428 (inflexion), 448, 478 nm, and of pigment B, amorphous red power, m.p. 180–185°, λ_{max} (petroleum ether) 278, 340 (*cis*-peak), 425 (inflexion), 446, 473 nm. Pigment C was purified by chromatography on silicic acid using 10% acetone in benzene as developing solvent, and isolated as a dark-red amorphous solid, m.p. 200° (dec.), λ_{max} (petroleum ether) 278, 340 (*cis*-peak), 425 (inflexion), 445, 475 nm.

Pigment A was proved to be identical to isorenieratene[1] (I) by direct comparison with an authentic sample[4]. Pigments B and C were soluble in ethanolic potassium hydroxide and showed a hydroxyl band in the IR at 3590 cm^{-1} (methylene chloride solution). On treatment with acetic anhydride and pyridine, pigments B and C were converted to a monoacetate ($C_{42}H_{50}O_2$, m.p. 156–158°) and a diacetate ($C_{44}H_{52}O_4$, m.p. 182–184°) respectively, both derivatives showing UV and visible spectrum identical to those displayed by I (no *cis*-peak), and a phenolic acetate band at 1758 cm^{-1} in the IR (KBr pellets). Treatment of the acetyl derivative of pigment B with chromic anhydride in benzene-acetic acid gave crocetindial[5], 2, 3, 6-trimethylbenzaldehyde[6], and 2, 3, 6-trimethyl-4-acetoxybenzaldehyde[7], as main breakdown products. All compounds were identified by direct comparison (UV, visible, and IR-spectra, mixed m.p., chromatographic behaviour) with authentic samples prepared by synthesis. Similarly, oxidation of the diacetyl derivative of pigment C gave crocetindial and 2, 3, 6-trimethyl-4-acetoxybenzaldehyde as major reaction products. Structures II and III could therefore be written for pigments B and C.

The new carotenoids B (3-hydroxyisorenieratene) and C (3, 3′-dihydroxyisorenieratene) have been subsequently prepared by chemical synthesis. The Wittig reaction of crocetindial with triphenyl-2, 3, 6-trimethylbenzylphosphonium bromide[8] and triphenyl-[2, 3, 6-trimethyl-4-(2′-tetrahydropyranyloxy)]-benzylphosphonium bromide as reagents was used. For the preparation of the latter reagent, 2, 3, 6-trimethyl-4-hydroxybenzaldehyde[9] was converted to the 4-(2′-tetrahydropyranyloxy) derivative, then reduced with lithium aluminum hydride to 2, 3, 6-trimethyl-4-(2′-tetrahydropyranyloxy)-benzylalcohol, m.p. 85°, which was in turn converted to the phosphonium bromide by the usual procedure[10]. When crocetindial was allowed to react with triphenyl-2, 3, 6-trimethyl-4-(2′-tetrahydropyranyloxy)-benzylphosphonium bromide in the presence of *n*-butyl lithium, symmetric 3, 3′-ditetrahydropyranyloxyisorenieratene, $C_{50}H_{64}O_4$, m.p. 185–187°, was obtained. Removal of the protecting group by acid treatment afforded III (overall yield 60%). When the Wittig reaction was repeated starting with a mixture of both phosphonium bromides, a product was obtained from which, after removal of the tetrahydropyranyl group, 3-hydroxy-isorenieratene (II) was isolated in 30% yield, together with lower amounts of I and III. The synthetic compounds II and III displayed the same visible and IR-spectra, and identical chromatographic behaviour, also after acetylation, as natural pigments B and C.

The peptide antibiotic histidomycin[11] was isolated from the filtered broth by absorption-elution on ion exchange resins followed by molecular sieves fractionation. The identification of the antibiotic was performed on the basis of its chemical and biological properties, and of a direct comparison with an authentic sample[12]. The substance was responsible for the antibacterial activity displayed by culture liquids of *S. mediolani*[13].

Riassunto. Dal micelio di *Streptomyces mediolani* n. sp., coltivato in condizioni aereate e sommerse, sono stati isolati isorenieratene e due nuovi carotenoidi fenolici. In base ai risultati della demolizione ossidativa sono state attribuite ai due nuovi composti le strutture corrispondenti al 3-ossiisorenieratene e 3, 3′-diossiisorenieratene. È stata inoltre realizzata la sintesi dei due pigmenti con un procedimento comportante una condensazione, secondo Wittig, fra crocetindialdeide e gli appropriati alogenuri di benzilfosfonio.

F. Arcamone, B. Camerino,
E. Cotta, G. Franceschi, A. Grein,
S. Penco and C. Spalla

Istituto Ricerche Farmitalia,
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[4] We are indebted to Prof. B. C. Weedon, Queen Mary College, London, for a sample of isorenieratene.

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[12] We are indebted to Dr. E. A. Kaczka, Merck Sharp and Dohme, Rahway, USA, for a sample of histidomycin.

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