hope of obtaining chemical "peptidases" more selective than enzymes.

(8) Visiting Scientist at the National Institutes of Health on leave of absence from the Weizmann Institute, Rehovoth, Israel.

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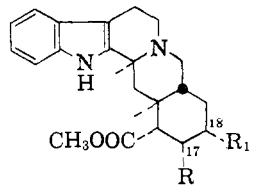
RECEIVED JUNE 23, 1958

MICROBIOLOGICAL TRANSFORMATION OF RAUWOLFIA ALKALOIDS

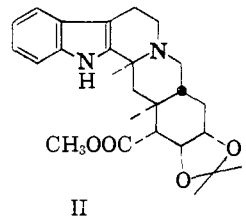
Sir:

In recent years the study of the actions of microorganisms on steroids has led to the discovery of many new transformations difficult to carry out by chemical means, and to production of new important pharmacologically active compounds. A study of the action of microörganisms on alkaloids particularly of the indole type, appeared especially attractive since the susceptibility of the indole moiety to attack by many chemical oxidative processes limits changes that may be made in other parts of the molecule.[1] We now wish to report the 18α-hydroxylation of yohimbine and α-yohimbine by *Streptomyces aureofaciens* (ATCC 11834) and *Streptomyces rimosus* (NRRL 2234).

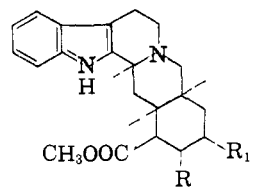
The Streptomyces cultures were grown in a medium containing 1.5% soybean meal, 2.5% glucose, 0.25% calcium carbonate and 0.5 mg./ml. of yohimbine hydrochloride. After incubation at 25° on a rotary shaker set at 280 r.p.m. with a 2-inch stroke for 1-2 weeks, the conversion could be demonstrated by subjecting the fraction extractable with chloroform at *p*H 9-10 to paper chromatographic analysis using the solvent system *i*-amyl alcohol–carbon tetrachloride–propionic acid (75:60:2) against water vapor equilibrated Whatman No. 1 paper.[2] The product appeared as a spot detectable by fluorescence, ultraviolet absorption and ferric ferricyanide spray, with an R_f value of 0.15 (R_f of yohimbine, 0.50). A control fermentation with no added yohimbine and an uninoculated medium containing yohimbine did not give rise to this product. By using the same extraction and chromatographic procedure, the chloroform extractable material from 4.5 l. of fermentation broth (45 flasks) was separated on twelve sheets of paper 10.5 inches wide. The appropriate band was eluted with methanol, and the eluate was crystallized from ethyl acetate–acetone to give 117 mg. of colorless cubes, m.p. 252-252.5°, $[\alpha]D+37°$ (methanol. The new substance was assigned structure Ib on the basis of the evidence: analysis gave the composition, $C_{21}H_{26}O_4N_2$ (found: C, 67.93; H, 7.07; CH_3O, 8.7; eq. wt. (perchloric acid), 367), corresponding to the addition of one oxygen atom to yohimbine (Ia). That the oxygen was present as a secondary hydroxyl group was shown by the formation of a diacetate (Ic) (m. p. 307-307.5°, $[\alpha]D-39°$ (chloroform); found: C, 66.44; H, 6.72; CH_3CO, 19.7) on treatment with pyridine and acetic anhydride. The substance gave an acetonide (II) (m. p. 258-259°, found : C, 70.40; H, 7.43) by reaction with acetone–perchloric acid, indicating that the new hydroxyl group was situated at C-18 and *cis* to the C-17 hydroxyl function. The presence of a 1,2-glycol system was evidenced by the formation of a bis-2,4-dinitrophenylhydrazone derivative after treatment of Ib with periodic acid.



Ia, R = OH, R_1 = H
Ib, R = R_1 = OH
Ic, R = R_1 = CH_3COO–



II



IIIa, R = OH, R_1 = H
IIIb, R = OH, R_1 = OH
IIIc, R = R_1 = CH_3COO–

When the same fermentation procedure was applied using α-yohimbine[3] (IIIa) as substrate, a new substance moving with an R_f of 0.17 (R_f of α-yohimbine 0.45) was detected. The product was isolated from the fermentation in the manner described above for 18α-hydroxyyohimbine. The hydrochloride crystallized in colorless needles from methanol–hydrochloric acid, m. p. 288-290° ($C_{21}H_{26}O_4N_2 \cdot HCl \cdot O \cdot 5H_2O$, found: C, 60.39; H, 7.00). It was shown to be 18α-hydroxy-α-yohimbine (IIIb) since it formed a diacetate (IIIc), m.p. 278-279°, ($[\alpha]D-14.5°$ (chf.), found C, 65.86; H, 6.58; CH_3CO, 19.47), an acetonide, m. p. 144-146°, and reacted with periodic acid to give a *bis*-2,4-dinitrophenylhydrazone.

(3) A. LeHir, M. M. Janot and R. Goutarel, *Bull. soc. chim. France*, **20**, 1027 (1953).

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH — S. C. PAN
NEW BRUNSWICK, NEW JERSEY — FRANK L. WEISENBORN

RECEIVED AUGUST 1, 1958

STRUCTURE OF A NEW ANTIBIOTIC, PYOLUTEORIN

Sir:

A new antibiotic, pyoluteorin, has been isolated from cultures of *Pseudomonas aeruginosa*, T 359 and IFO 3455,[1] and shown to have a powerful antibacterial activity *in vitro*.[2]

As was previously described,[2] pyoluteorin (I) ($C_{11}H_7O_2NCl_2$) has these physical properties: m.p. 174-175°(dec.); $\lambda_{max.}^{EtOH}$ 255 mμ (ϵ 4,200), 310 (ϵ 13,000); $\lambda_{max.}^{Nujol}$ 3.02 μ (OH,NH), 6.14(conj. C=O); found: C, 48.48; H, 2.86; N, 5.11; Cl, 25.82; mol.wt. 268 (Rast method), no C-CH_3, no N-CH_3,

(1) Since completion of this work W. O. Godtfresen, *et al.*, *Experientia*, **14**, 88 (1958), have reported the microbiological hydroxylation of apoyohimbine, 3-epiapoyohimbine and 3-yohimbine methyl ether.

(2) W. T. Sokolski, S. Ullman, H. Koffler and P. A. Tetrault, *Antibiotics and Chemotherapy*, **4**, 1057 (1957).

(1) Inst. for Fermentation, Osaka, List of Cultures, 108 (1956).

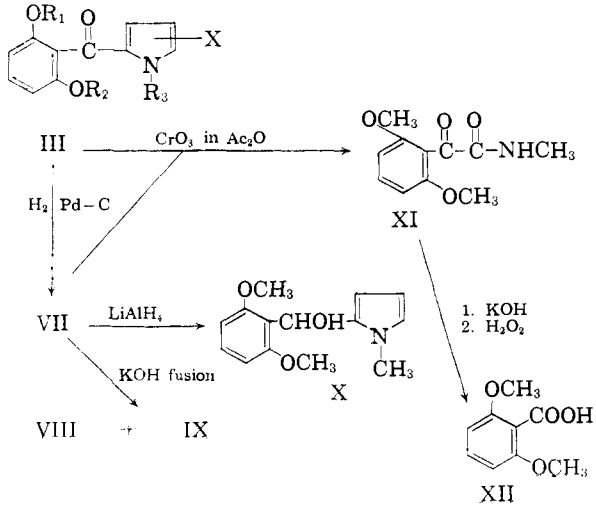
(2) R. Takeda, *J. Fermentation Technology*, Osaka, in press.

optically inactive. Acetylation of I with acetic anhydride in benzene gave an *O,O'*-diacetate (II) (m.p. 208°; $\lambda_{max.}^{Nujol}$ 3.09 μ (NH), 5.68, 5.77(ester C=O), 6.10 (conj. C=O); found: C, 50.53; H, 3.07; N, 3.83; Cl, 20.00; $COCH_3$,23.81). Methylation of I either with diazomethane or with dimethyl sulfate gave a trimethyl compound (III) (m.p. 135-136°; $\lambda_{max.}^{Nujol}$ 6.09 μ (conj. C=O), no OH-band, no NH-band; found: C, 53.28; H, 4.15; N, 4.63; Cl, 22.51; OCH_3, 19.49; N-CH_3, 4.44). On catalytic reduction of II in the presence of palladium-charcoal a dechloro compound (IV) (m.p. 110°; $\lambda_{max.}^{Nujol}$ 3.07 μ (NH), 5.70(ester C=O), 6.28(conj. C=O); found: C, 62.55; H, 4.63; N, 4.90) was obtained, which, on hydrolysis with potassium hydrogen carbonate, gave dechloropyoluteorin (V) (m.p. 142-143°, $\lambda_{max.}^{Nujol}$ 2.95, 3.18 μ (OH, NH), 6.28 (conj. C=O), Found: C, 64.75; H, 4.29; N, 6.67). The compound V was treated with diazomethane to yield an *O,O'*-dimethyl derivative (VI) (m.p. 187-188°; $\lambda_{max.}^{Nujol}$ 3.18 μ (NH), 6.18 (conj. C=O); found: C, 67.38; H, 5.45; N, 6.33; OCH_3, 26.55). III on hydrogenation over palladium-charcoal yielded trimethyldechloropyoluteorin (VII) (m.p. 137°; $\lambda_{max.}^{Nujol}$ 6.14 μ (conj. C=O), no OH-band, no NH-band; found: C, 68.68; H, 6.10; N, 5.98; OCH_3, 25.01; N-CH_3, 5.82).

Fusion of VII with potassium hydroxide resulted in the formation of 1,3-dimethoxybenzene (VIII) (b.p.$_{23}$ 105°; found: C, 69.82; H, 7.07) and N-methylpyrrole-2-carboxylic acid (IX) (m.p. 135°; found: C, 57.32; H, 5.42; N, 11.23).

The fact that alkali fusion furnished a product having a carboxyl group suggests that the carbonyl group is linked directly to both the benzene and the pyrrole rings. This was confirmed by reducing VII with lithium aluminum hydride, which led to 2,6-dimethoxyphenyl-N-methyl-2'-pyrrylmethanol (X) ($\lambda_{max.}^{EtOH}$ 241 mμ (ϵ 6,600) for the pyrrole ring, 271 mμ (ϵ 1,800) for the benzene ring; $\lambda_{max.}^{Nujol}$ 2.83 μ (OH), no conj. C=O band; not analyzed due to its instability). When VII was oxidized with chromium trioxide in acetic acid, the product was N-methyl-2,6-dimethoxybenzoylformamide (XI) (m.p. 143°; $\lambda_{max.}^{Nujol}$ 3.07 μ (NH), 5.87, 5.93, 5.99(conj. C=O), 6.56(NH); found: C, 59.34; H, 5.79; N, 6.33), which was also obtained by the similar treatment of III. XI was hydrolyzed with aqueous potassium hydroxide to the corresponding keto acid and the latter was further converted with hydrogen peroxide to 2,6-dimethoxybenzoic acid (XII) (m.p. 187°); which was identical with a synthetic sample. In view of the results obtained above, pyoluteorin must have the constitution I. The positions of the two chlorine atoms on the pyrrole ring will be clarified later.

$R_1 = R_2 = R_3 = H, X = Cl_2$ (I)
$R_1 = R_2 = COCH_3, R_3 = H, X = Cl_2$ (II)
$R_1 = R_2 = R_3 = CH_3, X = Cl_2$ (III)
$R_1 = R_2 = COCH_3, R_3 = H, X = H_2$ (IV)
$R_1 = R_2 = R_3 = H, X = H_2$, (V)
$R_1 = R_2 = CH_3, R_3 = H, X = H_2$ (VI)
$R_1 = R_2 = R_3 = CH_3, X = H_2$ (VII)



INSTITUTE FOR FERMENTATION ATTACHED TO THE TAKEDA PHARMACEUTICAL INDUSTRIES LTD. ROKURO TAKEDA
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RECEIVED JULY 14, 1958

THE RELEASE OF ZINC FROM CARBOXYPEPTIDASE AND ITS REPLACEMENT

Sir:

Carboxypeptidase is a zinc metalloenzyme and the metal is functional in enzymatic action.[1,2] 1,10-Phenanthroline (OP)[3] inhibits enzymatic activity and removes zinc from the protein[1]; spectral changes can be observed under such conditions.[4] We have now observed that zinc can be progressively removed by dialysis of carboxypeptidase at *p*H values between 5.5 and 3.4. As *p*H is lowered, increasing amounts of zinc are removed at an accelerating rate. Activity is abolished at a similar rate (CGP[3] as substrate). The correlation coefficient between metal content and activity is 0.90. Pertinent data are given in Table I.

TABLE I

THE LOSS OF ZINC AND PEPTIDASE ACTIVITY FROM CARBOXYPEPTIDASE IN ACID SOLUTION

Experimental conditions: 1.0*M* NaCl, 0.1*M* citrate, 2.8 mg./ml. protein, 0°; per cent. zinc and peptidase activity were measured after 48 hours dialysis at the given *p*H; $t_{1/2}$ gives the time required to reach 50% of the equilibrium values.

*p*H	Zn, % At equilibrium	Activity, % At equilibrium	$t_{1/2}$, hr. Zn	$t_{1/2}$, hr. Activity
5.46	..	86	..	25
5.22	68	71	20	24
4.98	52	76	11	11
4.48	30	46	12	13
3.92	17	19	8	8
3.38	5	8	3	2

Zinc also can be removed by dialysis against 2×10^{-3} *M* OP at *p*H 8.0,[5] 7.0 and 4.2. When OP is then removed by dialysis, and zinc is added back to the metal-free, inactive protein by dialysis at *p*H 7.0, its zinc content can be restored to 1 gram atom per mole with concomitant complete res-

(1) B. L. Vallee and H. Neurath, THIS JOURNAL, **76**, 5006 (1954).
(2) B. L. Vallee and H. Neurath, *J. Biol. Chem.*, **217**, 253 (1955).
(3) OP = 1,10-phenanthroline; CGP = carbobenzoxyglycyl-L-phenylalanine.
(4) B. L. Vallee, T. L. Coombs and R. J. P. Williams, THIS JOURNAL, **80**, 397 (1958).
(5) T. L. Coombs and B. L. Vallee, The Biophysical Society, Abstracts of 1958 Meeting, Cambridge, Mass., p. 20.