of a peptide or protein by N-bromosuccinimide "titration," an additional 1-2 moles of N-bromosuccinimide per mole of tryptophan is added for the controlled cleavage[6] of the C-tryptophyl bonds. Figure 1 summarizes experiments with model peptides. The general usefulness of the method was demonstrated with glucagon,[7,8] the crystalline hyperglycemic-glycogenolytic peptide from pancreas, containing only one tryptophan among 29 amino acids.[9] N-Bromosuccinimide leads to the liberation of a major new ninhydrin-positive peptide, giving positive platinic chloride reaction for methionine[10] and negative reactions for histidine and arginine. Its hydrolysis yielded aspartic acid, threonine, methionine and leucine. This tetrapeptide, which arises from the C-terminal sequence TRY-LEU-MET-ASP-THR, has been obtained by the action of chymotrypsin[11] and trypsin[12] on glucagon. However, the cleavage of glucagon by N-bromosuccinimide is more rapid (<1 min.) and more selective than that by any known peptidase. The new method is being applied to other proteins and peptides.

(6) *Cf.* A. Patchornik, W. B. Lawson and B. Witkop, THIS JOURNAL, **80**, 4748 (1958).

(7) A. Staub, L. Sinn and O. K. Behrens, *J. Biol. Chem.*, **214**, 619 (1955).

(8) We are greatly indebted to Dr. O. Behrens, The Lilly Research Laboratories, for his interest and a liberal sample.

(9) W. W. Bromer, L. G. Sinn and O. K. Behrens, THIS JOURNAL, **79**, 2807 (1957).

(10) G. Toennies and J. J. Kolb, *Anal. Chem.*, **23**, 823 (1951).

(11) W. W. Bromer, L. G. Sinn and O. K. Behrens, THIS JOURNAL, **79**, 2798 (1957).

(12) W. W. Bromer, A. Staub, L. G. Sinn and O. K. Behrens, *ibid.*, **79**, 2801 (1957).

(13) 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

THE USE OF NEIGHBORING GROUP EFFECTS FOR THE SELECTIVE CLEAVAGE OF PEPTIDE BONDS. I. ON THE MECHANISM OF OXIDATION OF β-SUBSTITUTED INDOLES WITH N-BROMOSUCCINIMIDE[1]

Sir:

When indole-3-propionic acid was treated with 3 moles of N-Bromosuccinimide in methanolic acetate buffer of *p*H 4.0, a neutral compound was obtained as colorless needles from methanol–water, m.p. 199.5-200.5°, $C_{11}H_8NO_3Br$ (calcd.: C, 46.83; H, 2.86; N, 4.97; Br, 28.33. Found: C, 46.70; H, 2.92; N, 4.92; Br, 28.46); $\lambda\lambda_{max}^{EtOH}$ 308, 260 mμ; $\lambda\lambda_{max}^{KBr}$ 5.62 (five membered lactone), 5.76 μ (oxindole). The data suggest the structure I of a spiro lactone of a dioxindole-3-propionic acid carrying a bromine, presumably in the 5-position. When the same reaction was carried out with indole-3-propionylglycine ($C_{13}H_{14}N_2O_3$, m.p. 159-160°; found: C, 63.39; H, 5.60; N, 11.21) up to 60% of glycine was liberated.[2] Table I summarizes the yields of liberated amines from the parent peptides or amides as a function of the length of the indole-β-side chain and of the nature and substitution of the amine and shows that optimal cleavage occurs with the propionic acid side chain where 1,5-interaction VIII and formation of a cyclic imino ether IX and hydrolysis[3] to a γ-lactone are possible. The imidole contribution is suppressed in the *p*-nitroanilide V and no cleavage occurs. 1,5-[4] and 1,6-interactions[5] have been observed in displacement reactions caused by participating amide groups and a close analogy exists in the reaction of N-bromosuccinimide with β-benzamidopropene.[6] 1,4-Interaction in indole-3-acetyl derivatives is negligible, while 1,6-interaction (IV) leads to less than 1/3 of free amine compared with III. The failure of N-bromosuccininiide to liberate much ethyl glycinate from the 2-hydroxytryptophan derivative VI, in contrast to the tryptophan derivative VII, points to a compound other than VIII as the true intermediate, possibly a β-bromoindolenine or β-bromoindolinol.[7]

No.	Compound	M.p., °C.	Yield of amine	
II	Ethyl indole-3-acetylglycinate	88	3	*a*
III	Ethyl indole-3-propionylglycinate	77–78	55	*a*
IV	Ethyl indole-3-butyrylglycinate	115–116	17	*a*
V	Indole-3-propion-*p*-nitroanilide	216–219	..	*b*
VI	Ethyl N-carbobenzyloxyoxytryptophylglycinate	170–173	13	*a*
VIII	Ethyl N-carbobenzyloxytryptophylglycinate	117	39	*a*

[a] Measured colorimetrically in a Beckman Model B spectrophotometer at 570 mμ with a glycine ethyl ester standard; lactone I does not interfere in this determination. Independent chromatographic analysis proved the presence of only one ninhydrin-positive material corresponding to the liberated amine. [b] No *p*-nitroaniline detected in the ultraviolet.

The concept of selective activation of inert peptide groups by making them participants in intramolecular displacement reactions raises the

(1) Presented in part at the Fourth International Congress of Biochemistry in Vienna, Sept. 1–6, 1958.

(2) A. Patchornik, W. B. Lawson and B. Witkop, THIS JOURNAL, **80**, 4747 (1958), Fig. 1.

(3) *Cf.* R. Kuhn and D. Weiser, *Angew. Chemie*, **69**, 371 (1957).

(4) *Cf.* O²,2′-cyclouridine: D. M. Brown, A. Todd and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

(5) *Cf.* O²,5′-cyclouridine: D. M. Brown, A. Todd and S. Varadarajan, *ibid.*, 868 (1957).

(6) L. Goodman and S. Winstein, THIS JOURNAL, **79**, 4788 (1957).

(7) α-Bromination is observed in non-aqueous systems: F. Troxler and A. Hofmann, *Helv. Chim. Acta*, **40**, 2161 (1957).

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

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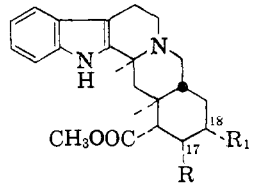
RECEIVED JUNE 23, 1958

MICROBIOLOGICAL TRANSFORMATION OF RAUWOLFIA ALKALOIDS

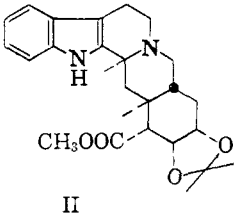
Sir:

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).

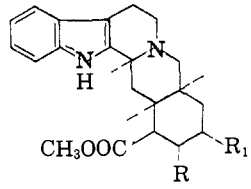
The Streptomyces cultures were grown in a medium containing 1.5% soybean meal, 2.5% glucose, 0.25% calcium carbonate and 0.5 mg./ml. of yohimbine hydrochloride. After incubation at 25° on a rotary shaker set at 280 r.p.m. with a 2-inch stroke for 1-2 weeks, the conversion could be demonstrated by subjecting 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).

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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.