ammonia by warming on a steam bath. The dark amber solution was clarified with Darco and, after cooling to room temperature, the pH was adjusted to 7–8 by the addition of 5 ml. of 9 *N* sulfuric acid. After overnight refrigeration the solid was collected, washed with cold water, and dried. The yellow prismatic rods weighed 5.5 g. (56% yield). Three more precipitations from ammonia, the last without Darco, afforded the analytical sample; no m.p. below 360° (lit. 355° dec.,[22] >360°[2]).

A sample of XV prepared by this route was shown to be identical with material prepared by thiation of authentic 4-amino-6-chloro-2-hydroxypyrimidine (XIV) obtained by acid hydrolysis of 4-amino-6-chloro-2 methylthiopyrimidine (XVIII).[2] This identity is based upon melting points, comparison of ultraviolet and infrared spectra, and behavior on paper chromatography.

Acknowledgment.—We wish to thank Dr. Janine Kint, Miss Dorothy H. Trites, and Mrs. Abby L. Sandler for technical assistance at various times during this investigation. We are indebted to Mr. James H. Gunnerson for the infrared and ultraviolet absorption spectra. Larger quantities of the following pyrimidines were obtained through the courtesy of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, and were prepared according to the procedures outlined in the Experimental section: 2,4-diamino-6-hydroxypyrimidine hemihydrate (Aldrich Chemical Co., Milwaukee, Wisconsin, and Francis Earle Co., Peekskill, New York), 2,4-diamino-6-chloropyrimidine (Aldrich), and 2,4-diamino-6-mercaptopyrimidine half-sulfate (Aldrich).

Notes

Synthesis of Indomethacin Metabolites

R. G. STRACHAN, M. A. P. MEISINGER, W. V. RUYLE, RALPH HIRSCHMANN, AND T. Y. SHEN

Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., Rahway, New Jersey

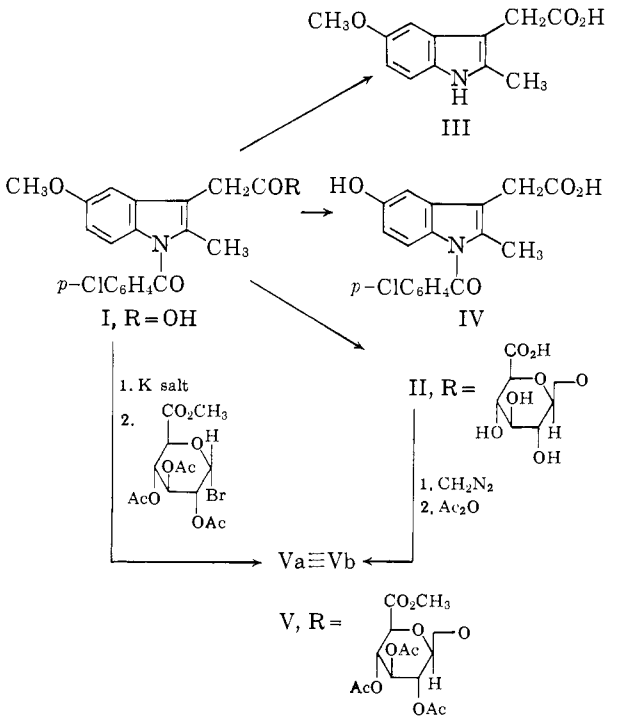
Received May 27, 1964

The isolation and characterization of the metabolites of indomethacin (I), a new nonsteroidal antiinflammatory agent,[1] have been reported recently.[2] In man, the acyl glucuronide of indomethacin (II) is rapidly excreted in the urine, whereas in several animal species the N_1-deacylated derivative III, the *O*-desmethyl analog IV, and the corresponding acyl glucuronides are major urinary metabolites.

During the study of indomethacin analogs the ease of hydrolytic removal of the N_1-aroyl group under mildly acidic or alkaline conditions was noted,[3] thus the *in vivo* N_1-deacylation of iodomethacin to the known acid III, previously described by Shaw,[4] was not totally unexpected.

For the preparation of the *O*-desmethyl analog IV, a preferential demethylation was effected in about 50% yield with pyridine hydrochloride at 180° without extensive concomitant N_1-deacylation.

The chemical lability of acyl glucuronides is well known.[5] Facile hydrolytic cleavage of the glycosidic linkage of II was also observed during its isolation.[2] To effect the synthesis of II, the potassium salt of indomethacin was converted to the ester glucuronide derivative Va by condensation with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate[6] in acetone. Several exploratory attempts to convert Va into II were abortive. Nevertheless, the crystalline derivative Va proved to be identical with a sample (Vb) prepared from the *in vivo* metabolite II by esterification and acetylation,[7] thus establishing the structure of II.



Experimental

1-*p*-Chlorobenzoyl-5-hydroxy-2-methyl-3-indolylacetic Acid (IV).—Indomethacin (I, 10 g., 0.028 mole) was added to 50 g. of molten pyridine hydrochloride (Eastman, practical grade) under nitrogen at 180°. The mixture was stirred at 180° for 0.25 hr., cooled slightly, and poured into 200 g. of ice. The solid product was filtered, dried, and digested successively with 50 ml. of methylene chloride and 150 ml. of ether. Recrystallization from acetone (75 ml.)–water (125 ml.) yielded 4.8 g. (50%) of IV, m.p. 208–210° dec.

Anal. Calcd. for $C_{18}H_{14}ClNO_4$: C, 62.89; H, 4.10; Cl, 10.31. Found: C, 63.02; H, 4.40; Cl, 10.32.

Compound IV was compared with a sample of the metabolite[2] as shown in Table I.

(Methyl 2′,3′,4′-Tri-*O*-acetyl-β-D-glucuronosyl)-1-*p*-chlorobenzoyl-5-methoxy-2-methyl-3-indolylacetate (V). A. From Indomethacin (Va).—1-*p*-Chlorobenzoyl-2-methyl-5-methoxy-2-indolylacetic acid (5 g., 0.014 mole) was suspended in 15 ml. of anhydrous methanol, and 32.8 ml. of a solution of 0.427 *N* (0.014

(1) T. Y. Shen, *et al.*, *J. Am. Chem. Soc.*, **85**, 488 (1963).
(2) R. E. Harman, M. A. P. Meisinger, G. E. Davis, and F. A. Kuehl, Jr., *J. Pharmacol. Exptl. Therap.*, **143**, 215 (1964).
(3) T. B. Windholz and T. Y. Shen, unpublished observation.
(4) E. Shaw, *J. Am. Chem. Soc.*, **77**, 4319 (1955).
(5) W. H. Fishman, "Chemistry of Drug Metabolism," Charles C Thomas, Springfield, Ill., 1961, pp. 95–98.
(6) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **111**, 347 (1935).
(7) I. A. Kamil, J. N. Smith, and R. T. Williams, *Biochem. J.*, **50**, 235 (1951).

TABLE I

	Metabolite[a]	Synthetic IV
M.p., C°.	213–215 dec.	208–210 dec.
Paper strip chromatography,[a] R_f	0.66	0.69
Thin layer chromatography,[b] R_f	0.15	0.13
Infrared (KBr pellet)	Identical	

[a] Whatman 3MM paper, isopropyl alcohol–15 *N* ammonium hydroxide–water (v./v. 8:1:1), visualized on an ultraviolet scanner. [b] Silica gel G, HOAc–$CHCl_3$ (v./v. 5:95), visualized by iodine vapor.

equiv.) of potassium *t*-butoxide in *t*-butyl alcohol was added dropwise. The solid dissolved slowly and at the end of the addition the solution was clear and neutral. The yellow solution was concentrated to dryness *in vacuo*, dissolved in the minimum amount of refluxing acetone, and placed in the refrigerator overnight. The resultant crystals were filtered, washed with a small amount of cold acetone, and dried, yielding 4.2 g. of the potassium salt of indomethacin. A 4-g. aliquot (0.0101 mole) of the salt was dissolved in the minimum amount of refluxing acetone and the resulting solution was treated with 4.1 g. (0.0103 mole) of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate dissolved in acetone. The solution was refluxed for 2 hr. in a nitrogen atmosphere. The color of the solution changed from yellow to maroon. After standing at room temperature overnight, the solution was concentrated to dryness and the residue was dissolved in methylene chloride. The solution was filtered, extracted three times with an equal volume of a saturated solution of $NaHCO_3$ with water, and dried over $MgSO_4$. The solution was concentrated to yield a viscous yellow oil, which was crystallized from ether–hexane to yield 2.0 g. of V, m.p. 150–151°; $\lambda_{max}^{CHCl_3}$ 1755, 1673 (C=O), 1585 (aromatic), and 1200–1235 cm.$^{-1}$.

Anal. Calcd. for $C_{32}H_{32}ClNO_{13}$: C, 57.79; H, 4.85; Cl, 5.33; N, 2.11. Found: C, 57.71; H, 4.59; Cl, 5.12; N, 2.06.

B. From Indomethacin Glucuronide (Vb).—A 13.5-mg. sample of indomethacin glucuronide isolated from rabbit urine[2] was dissolved in 1 ml. of methanol and cooled in an ice bath, and excess ethereal diazomethane was added. After 30 min. at room temperature, the solvent was removed from the reaction mixture with a stream of nitrogen. The amorphous residue was taken up in 1 ml. of 50% pyridine in acetic anhydride and held at 4° overnight. The solution was then poured into 5 ml. of ice water and stirred a few minutes at room temperature to decompose excess reagent. The precipitated solid was centrifuged and taken up in ether, and the ether solution was separated from traces of water by centrifugation. The crude derivative obtained by evaporating the ether was chromatographed on silica gel G (thin layer technique) using ethyl acetate as the developing solvent. Material at R_f 0.83 showed the same R_f and yellow fluorescence in ultraviolet light as the synthetic sample Va. This area was removed from the glass plate, the silica gel was extracted with ethyl acetate, and the crude derivative again was chromatographed on silica gel G using chloroform as the developer. The yellow fluorescence was observed at R_f 0.05, again parallel with the synthetic sample. The material was recovered with ethyl acetate and crystallized from ether–hexane, m.p. 144–145°, alone or mixed with Va. The infrared spectra of the two samples were identical.

Regeneration of Antibiotic Activity by Deacetylation of N-Acetyl Derivatives of Deoxystreptamine-Containing Antibiotics

GERALD H. WAGMAN AND MARVIN J. WEINSTEIN

Department of Microbiology, Schering Corporation, Bloomfield, New Jersey

Received July 8, 1964

Gentamicin, the most recent member of the deoxystreptamine-containing group of antibiotics to be described, has been resolved into two closely related, active, antibiotic components by basic hydrolysis of the previously separated microbiologically inactive N-acetyl derivatives.[1] The fact that gentamicin is in the same chemical family as neomycin, kanamycin, and paromomycin led us to believe that by use of the same procedure the inactive acetyl derivatives of this group of compounds might also be converted back to the active antibiotics. We are now able to report the regeneration of antibacterial activity from the inactive N-acetyl derivatives of kanamycin, neomycin, and paromomycin by this method.

Each of the antibiotics described was obtained as the sulfate salt, converted to the free base by ion exchange utilizing Amberlite IRA400 resin[2] in the hydroxyl phase, and acetylated by the method described by Rinehart, *et al.*[3]

The N-acetyl derivatives were tested at 10 mg./ml. against *Staphylococcus aureus* ATCC 6538P and *Bacillus subtilis* ATCC 6633 and found to have no antibacterial activity. Ninhydrin reactions were also negative in all instances indicating complete acetylation of all reactive amino groups. The regeneration to the biologically active base was accomplished by dissolving 250 mg. of the N-acetyl compound in 25 ml. of water and adding 2.5 ml. of 50% w./w. NaOH (19 *N*). Each mixture was saponified at reflux temperature for 48 hr. Samples were taken at convenient time intervals, diluted 100-fold with 0.02 *N* H_2SO_4 to approximately pH 8.5, and antibiotic activity was measured by the appropriate microbiological assay.

The rates of regeneration of antibacterial activities from all four inactive N-acetyl derivatives are shown in Table I. The data indicate total regeneration of

TABLE I

RATE OF REGENERATION OF N-ACETYL DERIVATIVES OF GENTAMICIN, KANAMYCIN, NEOMYCIN, AND PAROMOMYCIN[a]

Reflux time, hr.	Antibiotic activity, γ/ml. × 10³ Gentamicin	Kanamycin	Neomycin	Paromomycin
0	0.0	0.0	0.0	0.0
1	0.94	1.3	0.92	0.80
3	2.6	2.9	2.0	2.1
6	5.4	4.4	3.5	3.3
24	7.7	7.4	5.0	4.9
30	9.0	6.2	6.3	6.4
48	9.4	4.8	6.5	5.2

[a] Conditions: boiling at reflux after addition of 10% by volume of 50% (w./w.) NaOH (19 *N*).

these antibiotics, within the errors of the assays, based on the assumption that the acetylation procedure affords theoretically pure N-acetyl compounds used as starting materials.

After determining optimum regeneration time from the data in Table I, batches of the N-acetyl compounds were prepared and hydrolyzed as previously described. At completion of refluxing, the reaction mixtures were neutralized with H_2SO_4, precipitates were separated by centrifugation, and the supernatant liquids were concentrated. Each concentrate was passed through a column of Amberlite IRA400 in the chloride form;

(1) M. J. Weinstein, G. M. Luedemann, E. M. Oden, G. H. Wagman, J. P. Rosselet, J. A. Marquez, C. T. Coniglio, W. Charney, H. L. Herzog, and J. Black, *J. Med. Chem.*, **6**, 463 (1963).

(2) A quaternary base anion-exchange resin sold by the Rohm and Haas Co.

(3) K. L. Rinehart, Jr., A. D. Argoudelis, W. A. Goss, A. Sobler, and C. P. Schaffner, *J. Am. Chem. Soc.*, **82**, 3938 (1960).