TABLE I

	Metabolite[a]	Synthetic IV
M.p., C°.	213–215 dec.	208–210 dec.
Paper strip chromatography,[c] 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

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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. Solder, and C. P. Schaffner, *J. Am. Chem. Soc.*, **82**, 3938 (1960).

TABLE II

ALKALINE STABILITY OF GENTAMICIN, KANAMYCIN, NEOMYCIN, AND PAROMOMYCIN[a]

Reflux time, hr.	Antibiotic activity, γ/ml. × 10³ Gentamicin	Kanamycin	Neomycin	Paromomycin
0	5.8	9.5	6.0	7.8
19	5.7	11.2	6.5	8.0
25	5.8	9.6	6.3	8.5
48	5.7	10.0	6.3	7.8

[a] Conditions: boiling at reflux after addition of 10% by volume of 50% (w./w.) NaOH (19 *N*). Kanamycin, 10 mg./ml. as base; others, 10 mg./ml. as sulfates.

the effluent was dried to a residue and repeatedly triturated with methanol to remove salts. The regenerated antibiotic hydrochlorides were compared with the antibiotic starting materials (also as hydrochlorides) in a variety of chromatographic systems and were found to have identical R_f values in each instance. Each regenerated compound and its parent were then reacetylated and chromatographed using the method of Pan and Dutcher[4] and in each case were found to be identical.

Hydrolysis experiments were carried out on each of the free antibiotics to determine if the bases were as stable as the N-acetyl derivatives used in these studies under the same reaction conditions. The high stability of the free bases under these rigorous conditions is confirmed by the maintenance of microbiological activity as summarized in Table II. These data demonstrate the stability of these antibiotics toward alkali even more dramatically than that reported by Leach, *et al.*,[5] who refluxed neomycin with excess barium hydroxide for 18 hr. with no decrease in activity.

The results obtained in the present study indicate that rigorous conditions of refluxing at high pH for long periods of time are required to cleave the acetyl groups from these antibiotic derivatives. Previous reports[6,7] on the inability to recover an active antibiotic by attempted deacetylation were probably due to inadequate hydrolytic conditions.

(4) S. C. Pan and J. O. Dutcher, *Anal. Chem.*, **28**, 836 (1956).
(5) B. E. Leach, W. H. DeVries, H. A. Nelson, W. G. Jackson, and J. S. Evans, *J. Am. Chem. Soc.*, **73**, 2797 (1951).
(6) R. L. Peck, C. E. Hoffhine, Jr., P. H. Gale, and K. Folkers, *ibid.*, **75**, 1018 (1953).
(7) J. D. Dutcher in "Neomycin: Its Nature and Practical Application," S. A. Waksman, Ed., Williams and Wilkins Co., Baltimore, Md., 1958, Chapter 7.

5,8-Isoquinolinediones. I. Synthesis of 5,8-Isoquinolinedione[1]

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The potential physiological activity of certain quinones, which are heterocyclic analogs of 1,4-naphthoquinone, has not been fully exploited. The biological activity of 6-(1-aziridinyl)-5,8-quinolinedione has been studied[2] and a number of substituted 5,8-quinolinediones have been reported to have physiological activity.[3-5] Although vitamin K antagonists have been the subject of several investigations,[6] no work has been done with the quinoline, quinoxaline, or isoquinoline analogs of 1,4-naphthoquinone. It was reported that 6-methyl-5,8-quinolinedione did not show any antihemorrhagic activity,[7] but no information could be found on the possible antivitamin K activity of this compound.

The recent interest in 5,8-quinolinediones led us to investigate the closely related 5,8-isoquinolinediones. The aim of our study was to explore the potential physiological activity of these compounds and also to compare the chemical reactivity of this system to that of the well-known 1,4-naphthoquinone system. Thus, we looked for a suitable route to synthesize first the unsubstituted compound, 5,8-isoquinolinedione.

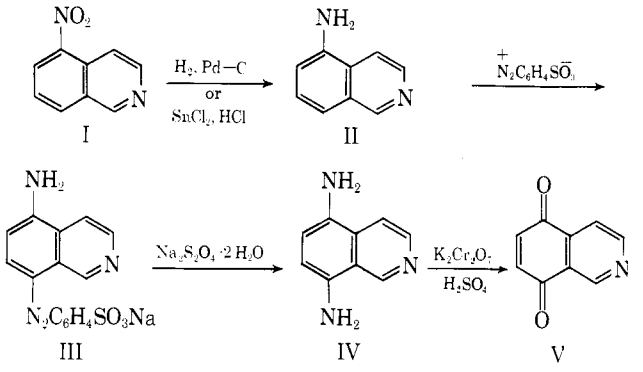
The synthesis of 5,8-isoquinolinedione by the oxidation of 5-amino-8-hydroxyisoquinoline was attempted by Fieser and Martin,[8] but the desired compound was not obtained. The only product isolated was reported to be the hydrochloride of isoquinoline-5,8-hydroquinone. The authors suggested that this compound might have resulted from a disproportionation of the quinone first formed.

More recently, the preparation of some substituted 5,8-isoquinolinediones has been reported[9] but only the picrates of these compounds were characterized.

We have attempted the preparation of 5,8-isoquinolinedione by the oxidation of the corresponding 5,8-diamine and have been able to isolate the pure quinone in good yields.

The synthesis of 5,8-isoquinolinedione was accomplished according to Scheme I. The first step of the

SCHEME I



synthesis was the nitration of isoquinoline according to known directions.[10] The pure product was reduced.[11,12]

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(2) S. Petersen, W. Gauss, and E. Urbschat, *Angew. Chem.*, **67**, 217 (1955).
(3) Farbenfab. Bayer Akt. Ges., British Patent 856,505 (1960); *Chem. Abstr.*, **55**, 12430*f* (1961).
(4) Farbenfab. Bayer Akt. Ges., British Patent 874,770 (1959); *ibid.*, **56**, 5968*g* (1962).
(5) A. Wagner, W. Beck, and A. Diskus, Austrian Patent 221,311 (1962); *ibid.*, **57**, 9823*h* (1962).
(6) A. Burger, "Medicinal Chemistry," 2nd Ed., Interscience Publishers, Inc., New York, N. Y., 1960, pp. 221–230, 650–654.
(7) A. Ansbacher, *Proc. Soc. Exptl. Biol. Med.*, **46**, 421 (1941).
(8) L. F. Fieser and E. L. Martin, *J. Am. Chem. Soc.*, **57**, 1840 (1935).
(9) M. Lora-Tamayo, R. Madroñero, and M. Stud, *Chem. Ber.*, **95**, 2176 (1962).
(10) C. G. KeFévre and R. J. W. LeFévre, *J. Chem. Soc.*, 1470 (1935).
(11) J. J. Craig and W. E. Cass, *J. Am. Chem. Soc.*, **64**, 783 (1942).
(12) F. W. Bergstrom and R. E. Paterson, *J. Org. Chem.*, **10**, 481 (1945).