acid salt, also showed inhibition against Ca755, and another, the *m*-aminobenzenesulfonic acid salt, possessed antitumor activity against the S180 mouse tumor system with the latter compound showing confirmed activity. Table II lists the antitumor testing data for the three active compounds, supplied by the Cancer Chemotherapy National Service Center.

Steroids. XXX.[1a] Some Indolocholestanes and Indoloandrostanes[1b,c]

NORMAN J. DOORENBOS[1d] AND MU TSU WU

School of Pharmacy, University of Maryland, Baltimore, Maryland 21201

Received September 13, 1967

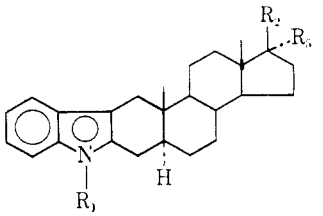
Interest in steroids to which heterocyclic rings are fused or attached developed in our laboratory[2] during a study of heterocyclic steroids of potential therapeutic value. One derivative of this type, 1′,4′,5′,6′-tetrahydropyrimidino[4,3-*a*]-5-cholestene,[2d] possesses significant antimicrobial,[3] antiinflammatory, hypocholesterolemic, hypotensive, and diuretic activities.[2d] Another derivative of this type, pyrazolo[3,2-*c*]-17α-methyl-5α-androstan-17β-ol,[4] possesses a high degree of separation of anabolic from androgenic activity and has been in clinical use for several years. Its discovery stimulated the synthesis of analogous heterocyclic steroids in several laboratories.[5] We wish to report the synthesis and preliminary biologic study of some indolocholestanes and indolandrostanes.

Doree[6a] obtained indolo[3,4-*b*]-5β-cholest-3-ene in 1909 when he attempted to prepare the phenylhydrazone of 5β-cholestan-3-one (I) by reaction in hot glacial acetic acid. In 1935, indolo[3,2-*b*]-5α-cholest-2-ene (II) was synthesized in a similar manner.[6b] Fusion of the indole ring at positions 2,3 in the 5α series and 3,4 in the 5β series was confirmed recently by identifying the ozonolysis products of these compounds.[6c]

Indolo[3,2-*b*]-17α-methyl-5α-androst-2-en-17β-ol (VI) was prepared by a procedure similar to that used by Doree in the synthesis of indolocholestanes. The N-methyl derivatives (III, VII, and VIII) of II, VI, and I were prepared similarly by the reaction of N-methyl-N-phenylhydrazine with the appropriate steroid ketone (Tables I and II). Identical products were obtained in somewhat lower yields by adding methyl iodide to solutions of II, VI, or I and sodamide in dry dioxane–liquid ammonia. N-Aminoindolo[3,2-*b*]-5α-cholest-2-ene (V) and N-aminoindolo[3,4-*b*]-5β-cholestene (X) were prepared from II and I by nitrosation and $LiAlH_4$ reduction. X was characterized further by reaction with acetic anhydride, N-acetamidoindolo-[3,4-*b*]-5β-cholest-3-ene (XI) being obtained.

Each of these steroids has the characteristic absorption of the indole ring at approximately 13.50 μ.[7] Each nitroso derivative has a strong peak at 6.95 μ which is consistent with NN=O.[8]

TABLE I



Compd	R_1	R_2	R_3	Method[a]	Yield, %	Solvent of recrystn	Mp, °C[b]	$[\alpha]^{25}D$, deg[c]	Formula	Analyses[d]
III	CH_3	C_8H_{17}	H	A	99	C_6H_6–MeOH	203–205	+64.0	$C_{34}H_{51}N$	C, H, N
IV	NO	C_8H_{17}	H	B	88	C_6H_6–petr ether (bp 30–60°)	130–132	+103.8	$C_{33}H_{48}N_2O$	C, H, N
V	NH_2	C_8H_{17}	H	C	89	C_6H_6–MeOH	223–224	+86.8	$C_{33}H_{50}N_2 \cdot H_2O$	H, N; C[e]
VI	H	OH	CH_3	D	70	MeOH–H_2O	233–234	+39.4	$C_{26}H_{35}NO$	H, N; C[f]
VII	CH_3	OH	CH_3	E	80	MeCN–MeOH	171–173	+48.3	$C_{27}H_{37}NO$	C, H, N

[a] Prepared as follows: A, treatment of 5α-cholestan-3-one with a sixfold excess of N-methyl-N-phenylhydrazine in HOAc at 95°; B, treatment of indolo[3,2-*b*]-5α-cholest-2-ene[6b] in HOAc–dioxane at 10° with a concentrated H_2O solution of KNO_2 in 10% excess; C, treatment of IV with $LiAlH_4$; D, treatment of 17α-methyl-5α-androstan-17β-ol-3-one with phenylhydrazine in HOAc at 95°; E, treatment of 17α-methyl-5α-androstan-17β-ol-3-one with N-methyl-N-phenylhydrazine in HOAc at 95°. [b] Melting points were taken on a Fisher-Johns apparatus and are corrected. [c] *c* 0.5, $CHCl_3$. [d] Analytical results for elements indicated were within ±0.4% of the theoretical values unless indicated otherwise. [e] C: calcd, 80.43; found, 79.93. [f] C: calcd, 82.71; found, 83.22.

Biological Testing.[9]—Each indole steroid (I-XI) described in this paper was screened for antimicrobial activity against gram-negative bacteria (*Escherichia coli*, *Salmonella typhosa*, and *Brucella abortus*), gram-

(1) (a) For paper XXIX in this series, see N. J. Doorenbos and R. K. Sharma, *J. Chromatog.*, **29**, 393 (1967). (b) Presented in part before the Division of Medicinal Chemistry at the 141st National Meeting of the American Chemical Society, Washington, D. C., March 29, 1962. (c) Supported in part by a grant from Smith Kline and French Laboratories. (d) Address inquiries to N. J. D. at the School of Pharmacy, University of Mississippi, University, Miss. 38677.

(2) (a) N. J. Doorenbos and C. P. Dorn, *J. Pharm. Sci.*, **50**, 271 (1961); (b) N. J. Doorenbos and C. P. Dorn, *ibid.*, **51**, 414 (1962); (c) N. J. Doorenbos and C. P. Dorn, *ibid.*, **54**, 1219 (1965); (d) N. J. Doorenbos and M. T. Wu, *ibid.*, **54**, 1290 (1965); (e) N. J. Doorenbos and A. P. Shroff, *Steroids*, **5**, 399 (1965); (f) N. J. Doorenbos and L. Milewich, *J. Org. Chem.*, **31**, 3193 (1966).

(3) R. F. Smith, D. E. Shay, and N. J. Doorenbos, *J. Pharm. Sci.*, **53**, 1214 (1964).

(4) R. O. Clinton, A. J. Manson, F. W. Stonner, A. L. Beyler, G. O. Potts, and A. Arnold, *J. Am. Chem. Soc.*, **81**, 1513 (1959).

(5) P. de Ruggieri, G. Gandolfi, U. Guzzi, D. Chiaramonti, and C. Ferrari, *Farmaco* (Pavia), *Ed. Sci.*, **20**, 280 (1965).

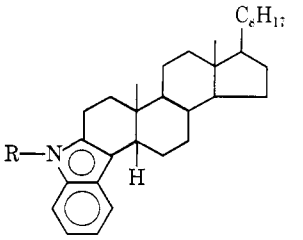
(6) (a) C. Doree, *J. Chem. Soc.*, 638 (1909); (b) C. Doree and V. A. Petrow, *ibid.*, 1391 (1935); (c) Y. Ban and Y. Sato, *Chem. Pharm. Bull.* (Tokyo), **13**, 1073 (1965).

(7) A. F. Chaplin, D. H. Iley, and J. Honeyman, *J. Chem. Soc.*, 3191 (1959).

(8) R. N. Jones, Infrared Spectra of Organic Compounds, NRC Bulletin No. 6, National Research Council, Ottawa, Canada, 1959.

(9) (a) Antimicrobial assays were conducted by Dr. Rodney F. Smith in our laboratory. (b) The results of the endocrine assays were provided by Dr. Kerwin and Dr. Saunders of Smith Kline and French Laboratories.

TABLE II



Compd	R	Method[a]	Yield, %	Recrystn solvent	Mp, °C[c]	$[\alpha]^{25}D$, deg[d]	Formula	Analyses[e]
VIII	CH_3	A	90	C_6H_6–MeOH	147–149	+137.4	$C_{34}H_{51}N$	C, H, N
IX	NO	B	71	EtOEt	146–148 dec	+132.2	$C_{33}H_{48}N_2O$	C, H, N
X	NH_2	C	89	*b*	164–165	+156.0	$C_{33}H_{50}N_2$	C, N; H[f]
XI	$NHCOCH_3$	D	79	MeOH	85–89	+118.8	$C_{35}H_{52}N_2O$	C, H, N

[a] Prepared as follows: A, treatment of 5β-cholestan-3-one with N-methyl-N-phenylhydrazine in HOAc at 95°; B, treatment of indolo[3,4-*b*]-5β-cholest-3-ene[6a] in HOAc–dioxane at 10° with a concentrated H_2O solution of KNO_2 in 10 *M* excess; C, treatment of IX with $LiAlH_4$; D, treatment of X with Ac_2O. [b] Purified by trituration with MeOH. [c] Melting points were taken on a Fisher-Johns apparatus and are corrected. [d] *c* 0.5, $CHCl_3$. [e] See footnote *d*, Table I. [f] H: calcd, 10.62; found, 10.15.

positive bacteria (*Staphylococcus aureus, Sarcina lutea,* and *Streptococcus pyogenes*), yeasts (*Candida albicans* and *Saccharomyces cerevisae*), and molds (*Sporotrichum schenckii* and *Trichophyton mentagrophytes*). A gradient plate technique[10] was used. None of these steroids exhibited activity.

Neither VI nor VII exhibited anabolic, androgenic, antianabolic, or antiandrogenic activity upon subcutaneous administration to castrate male rats as determined by its effect alone and in combination with testosterone upon seminal vesicles and levator ani muscle. II–VII were inactive upon subcutaneous administration to castrate male rats in a general endocrine screen.

(10) R. F. Smith, D. E. Shay, and N. J. Doorenbos, *J. Bacteriol.*, **85**, 1295 (1963).

Penicillins from 3- and 5-Phenylisothiazole-4-carboxylic Acids and Alkoxy Derivatives

R. G. MICETICH AND R. RAAP

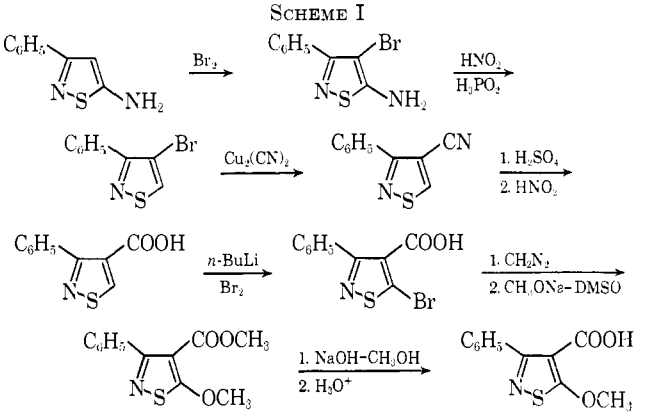
R and L Molecular Research Ltd., Edmonton, Alberta, Canada

Received July 25, 1967

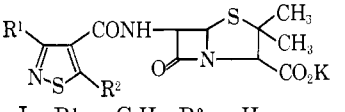
A study of penicillins derived from sterically hindered carboxylic acids[1,2] has shown that such penicillins show a high degree of resistance to the action of the enzyme penicillinase. This study has resulted in clinically useful penicillins such as 2,6-dimethoxyphenylpenicillin (methicillin), 3-phenyl-5-methyl-4-isoxazolylpenicillin (oxacillin), 3-*o*-chlorophenyl-5-methyl-4-isoxazolylpenicillin (cloxacillin), and 2-biphenylylpenicillin (diphenicillin). The analogy between the isoxazole and isothiazole[3] ring suggested that penicillins derived from hindered isothiazolecarboxylic acids should also show resistance to the action of penicillinase. Some penicillins of this type were recently reported by Grant, Pain, and Slack.[4] The present paper reports the synthesis and antibacterial activity of some phenyl- and alkoxy-substituted 4-isothiazolylpenicillins.

Chemistry.—3-Phenylisothiazole-4-carboxylic acid and 5-methoxy-3-phenylisothiazole-4-carboxylic acid were synthesized from 5-amino-3-phenylisothiazole[5] by the sequence of reactions illustrated in Scheme I.

SCHEME I



5-Phenylisothiazole-4-carboxylic acid was prepared from 3-amino-4-bromo-5-phenylisothiazole[6] by reductive deamination, followed by cyanation and hydrolysis. 3-Alkoxy-4-bromo-5-phenylisothiazoles[6] were converted to 3-alkoxy-5-phenylisothiazole-4-carboxylic acids in the same manner *via* the cyanides. The carboxylic acids were converted to the corresponding acid chlorides, which upon reaction with 6-aminopenicillanic acid and subsequent treatment with potassium 2-ethylhexonate provided the penicillins Ia–e.



Ia, $R^1 = C_6H_5$; $R^2 = H$
b, $R^1 = C_6H_5$; $R^2 = OCH_3$
c, $R^1 = H$; $R^2 = C_6H_5$
d, $R^1 = OCH_3$; $R^2 = C_6H_5$
e, $R^1 = OC_2H_5$; $R^2 = C_6H_5$

(1) J. C. Hanson, J. H. C. Nayler, T. Taylor, and P. H. Gore, *J. Chem. Soc.*, 5984 (1965), and preceding publications.

(2) A. W. Chow, N. M. Hall, J. R. E. Hoover, M. M. Dolan, and R. J. Ferlunto, *J. Med. Chem.*, **9**, 551 (1966), and preceding publications.

(3) R. Slack and K. R. H. Wooldridge, *Advan. Heterocyclic Chem.*, **4**, 107 (1965).

(4) M. S. Grant, D. L. Pain, and R. Slack, *J. Chem. Soc.*, 3842 (1965).

(5) J. Goerdeler and H. W. Pohland, *Chem. Ber.*, **94**, 2950 (1961).

(6) J. G. Goerdeler and W. Mittler, *ibid.*, **96**, 944 (1963).