DMF–H_2O to give 4.0 g (65%) of the product as an off-white solid, mp 297–299 °C.

References and Notes

(1) This is paper 44 of a series on antimalarial drugs. For paper 43, see J. Johnson, E. F. Elslager, and L. M. Werbel, *J. Heterocycl. Chem.*, **16**, in press (1979).
(2) This investigation was supported by the U.S. Army Medical Research and Development Command Contract DA-49-193-MD-2754. This is contribution No. 1542 to the Army Research Program on Malaria.
(3) Deceased June 21, 1973.
(4) E. F. Elslager, Proceeding of the International Symposium on Medicinal Chemistry, 4th, J. Maas, Ed., Elsevier, Amsterdam, 1974, pp 227–270.
(5) E. F. Elslager, P. Jacob, J. Johnson, L. M. Werbel, D. F. Worth, and L. Rane, *J. Med. Chem.*, **21**, 1059 (1978).
(6) E. F. Elslager, J. Davoll, P. Jacob, A. M. Johnson, J. Johnson, and L. M. Werbel, *J. Med. Chem.*, **21**, 639 (1978).
(7) S. Oae, Y. Ohnishi, S. Kozuka, and W. Tagaki, *Bull. Chem. Soc. Jpn.*, **39**, 364 (1964).
(8) The parenteral antimalarial screening in mice was carried out by Dr. Leo Rane, and test results were provided through the courtesy of Dr. T. R. Sweeney and Dr. E. A. Steck of the Walter Reed Army Institute of Research.
(9) For a description of the test method, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(10) Parenteral antimalarial screening against *P. gallinaceum* in chicks was carried out by Dr. Leo Rane at the University of Miami, and test results were supplied through the courtesy of Dr. T. R. Sweeney and Dr. E. A. Steck of the Walter Reed Army Institute of Research.
(11) For a description of the test method, see L. Rane, and D. S. Rane, *Proc. Helminthol. Soc. Wash.*, **39**, 283 (1972).
(12) Oral antimalarial testing was performed by Dr. Paul E. Thompson and colleagues in the laboratories of Parke-Davis and Co.
(13) These tests were performed by Dr. L. H. Schmidt: L. H. Schmidt, *W.H.O., Tech. Rep. Ser.*, **no. 529** (1973); L. H. Schmidt, *Trans. R. Soc. Trop. Med. Hyg.*, **67**, 446 (1973). For a definition of the magnitude of RI, RIII drug resistance, see *W.H.O., Tech. Rep. Ser.*, **no. 375**, (1967); **529** (1973).
(14) P. E. Thompson, A. Bayles, and B. Olszewski, *Exp. Parasitol.*, **25**, 32 (1969).
(15) P. E. Thompson, A. Bayles, and B. Olszewski, *Am. J. Trop. Med. Hyg.*, **19**, 12 (1970).
(16) W. Szybalski, *Microb. Genet. Bull.*, **5**, 16 (1954).
(17) A. H. Webb and L. Washington, *Bacterial. Proc.*, **52** (1966).
(18) For a more complete description of such antimetabolite studies, see: J. Davoll, A. M. Johnson, H. J. Davies, O. D. Bird, J. Clarke, and E. F. Elslager, *J. Med. Chem.*, **15**, 812 (1972).
(19) These studies were carried out by Dr. C. C. Smith and co-workers at the University of Cincinnati.
(20) These data were provided by Col. D. E. Davidson of the Walter Reed Army Institute of Research.

Notes

Syntheses and Biological Activities of 7β-Methyl Steroids

Wen-Hsiung Chiu* and Manfred E. Wolff

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143.

Received March 15, 1979

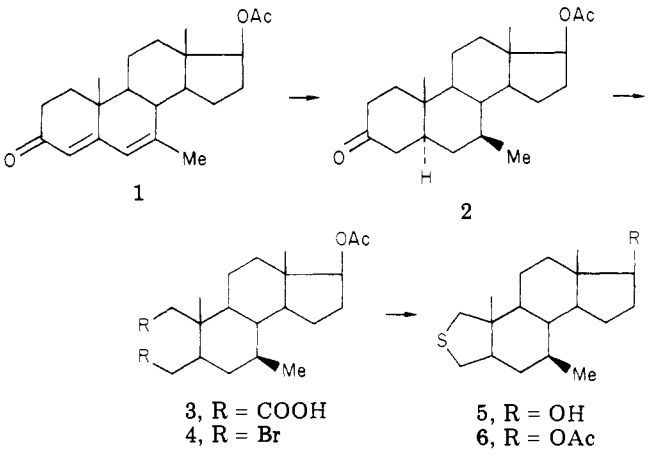
The preparation of 7β-methyl-5α-dihydrotestosterone acetate and its 2-thia-*A*-nor analogue is described. Biological evaluation shows that a 7β-Me largely decreases myotrophic–androgenic activity in both 5α-dihydrotestosterone and the 2-thia-*A*-nor analogue. Testing for antitumor activity shows that the reduction in breast tumor weight was not significant for either compound, but the final tumor size in the animals treated with 7β-methyl-2-thia-*A*-nor steroid at 10 (mg/kg)/day was significantly reduced. The effects of 7β-Me steroids on the various organ weights are also described. The influence of 7β-Me substituent on the biological activities of androgens may be mediated through direct interaction of the substituent with the receptor surface in contact with the third dimension of the steroid molecule.

Breast cancer is the single most important cause of cancer deaths in women in the U.S.[1] Like cancer of other organs under hormonal control, breast cancer may respond to hormonal therapy;[2] androgen treatment, in the form of testosterone propionate, produces regressions in about 25% of all patients[3] but produces undesirable virilization. By introduction of a 7β-methyl group into methyltestosterone, an equally efficacious antineoplastic drug (calusterone)[4] lacking strong masculinizing properties is produced.

In a previous study,[5] using derivatives of 2-thia-*A*-nor-5α-androstan-17β-ol as probes of steroid–receptor interactions, we showed that enhancing groups, such as 7α-Me, known to be useful in carbocyclic steroids, such as testosterone and 5α-dihydrotestosterone (DHT), could be introduced into heterocyclic steroids, such as 2-thia-*A*-nor steroids, to give similar increases in myotrophic–androgenic activity. Since the introduction of a 7β-Me into methyltestosterone decreased myotrophic–androgenic activity while increasing antitumor activity, one might expect similar effects on introducing such a group into 5α-DHT or into 2-thia-*A*-nor steroid. For this reason, the preparation of 7β-methyl-5α-dihydrotestosterone acetate (**2**) and 7β-methyl-2-thia-*A*-nor-5α-androstan-17β-ol acetate (**6**) was undertaken.

6-Dehydro-7-methyltestosterone acetate (**1**) was prepared in good yield by a reported procedure.[6] Catalytic hydrogenation of **1** in acetic acid gave a product (**2**) which had a positive CD curve and a positive Cotton effect in the ORD. It was assigned the 5α configuration **2** on this basis. The assignment of a 7β-Me configuration to **2** was made on the basis of the catalytic hydrogenation of the dienone system in **2** which would be expected to proceed by cis addition of hydrogen to the α face and the fact that **2** was not identical with the 7α-Me epimer.[5] Under similar conditions, Beyler et al.[7] obtained a 7β-methyl-5α-androstan-3-one derivative in very good yield from the

0022-2623/79/1822-1257$01.00/0



corresponding 7-Me-$\Delta^{4,6}$-3-keto steroid.

Opening of ring A by CrO_3 oxidation gave dioic acid **3**, which via a modified Hunsdiecker reaction[8,9] afforded dibromide **4**. By cyclization in the presence of Na_2S with concomitant cleavage of the protecting group (17-OAc), the dibromide **4** gave the desired 7β-methyl-2-thia-*A*-nor steroid **5**. Acetylation of **5** with acetic anhydride in pyridine solution gave 7β-methyl-2-thia-*A*-nor-5α-androstan-17β-ol acetate (**6**).

Discussion

The data from the pharmacological testing[10,11] are displayed in Table I. As expected, the 7β-Me analogue (**2**) of 5α-DHT acetate was only weakly androgenic compared to 5α-DHT, whereas the 7β-Me thiasteroid (**6**) was devoid of androgenic activity. Since the equatorial 7β-Me is in the plane of the steroid molecule, an influence on the α- or β-face attachment of the receptor at C-7 is probably not involved. The large decrease in the myotrophic–androgenic activity of 7β-Me-substituted steroids is probably mediated through direct interaction of the substituent with the receptor surface in contact with the third dimension (or front side) of the steroid molecule. A three-dimensional attachment of the steroid–receptor interaction was previously postulated by Vida.[12]

The 7β-Me may also be important in connection with antitumor activity, since the introduction of this group into methyltestosterone (e.g., calusterone) enhanced the antitumor efficacy in the treatment of advanced female breast cancer while decreasing the androgenic activity.[4,13]

The results of testing for antitumor activity[14] are summarized in Tables II and III. At 10 (mg/kg)/day, the final body weight/initial body weight ratio indicates no real toxicity due to **2** or **6**. Reduction in tumor weight was not significant for either compound, but the final tumor size in animals treated with **6** was significantly reduced. At 100 (mg/kg)/day, both **2** and **6** increased tumor weight. However, it should be pointed out that calusterone also is inactive against a battery of animal tumors,[15] although it is significantly more effective than any other sex steroid employed in the treatment of advanced human female breast cancer.[13] From this it is believed that animal screens do not adequately predict activity against human female breast cancer. Because compound **6** was devoid of androgenic activity, it is still possible that this compound might demonstrate the desired separation of activities—retention or increase of antitumor efficacy against human female breast cancer without the undesirable hormonal effects.

Of particular interest are the effects on the various organ weights. Tumor growth is a stressful effect and one generally sees some adrenal hypertrophy, thymolysis, and loss of spleen weight in mammary tumor-bearing animals. At the dose level used, both test compounds and calusterone had an estrogenic effect on the uterus, with **6** exhibiting the least activity. Animals treated with 7β-Me carbocyclic steroids (including calusterone) had significantly smaller ovaries, adrenals, thymus glands, and pituitary glands. On the other hand, animals treated with 7β-Me-2-thia-*A*-nor steroid had larger spleens. Moreover, the thiasteroid appeared to have a protective effect on the thymus.

Experimental Section[16]

7β-Methyl-17β-hydroxy-5α-androstan-3-one Acetate (2). To a solution of 4.1 g (11.9 mmol) of **1** in 136 mL of acetic acid and 14 mL of acetic anhydride there was added 150 mg of 10% Pd on charcoal. The mixture was hydrogenated at 600 mmHg initial hydrogen pressure (Parr low-pressure hydrogenator) for 1 h at room temperature. TLC indicated the completion of hydrogenation. The catalyst was removed by filtration, and the solvents were evaporated under reduced pressure. The oily residue was chromatographed on silica gel, using 4–5% acetone in benzene (v/v) as eluent, to give 2.4 g (58%) of **2**, mp 126–128 °C. It was crystallized from ethyl alcohol to give the analytical sample: mp 128–130 °C; ORD (*c* 0.4, EtOH), 20 °C; $[\phi]_{350}$ +2080°, $[\phi]_{308}$ +4590°, $[\phi]_{284}$ 0°, $[\phi]_{270}$ -1040°, $[\phi]_{249}$ 0°; CD $[\theta]_{292}$ +3860. Anal. ($C_{22}H_{34}O_3$) C, H.

7β-Methyl-17β-hydroxy-2,3-seco-5α-androstane-2,3-dioic Acid Acetate (3). To a stirred solution of 2.08 g (6.0 mmol) of **2** in 40 mL of glacial acetic acid at 55 °C there was added a solution of 2.0 g (20 mmol) of CrO_3 in 6.0 mL of water and 6.0 mL of acetic acid. The mixture was stirred at 60 °C for 7 h. TLC showed the completion of the reaction. The mixture was poured into 1 L of ice–water saturated with NaCl, and the resulting precipitate was collected by filtration and washed with water. The pale greenish white solid was taken up in aqueous $NaHCO_3$ solution, and the aqueous solution was washed with ether. The aqueous solution was cooled, saturated with NaCl, and acidified with HCl. The white precipitate was collected, washed with water, and air dried to give 2.07 g of powder. Crystallization from aqueous methyl alcohol afforded 1.15 g (48%) of **3**, mp 208–211 °C. Recrystallization from EtOAc gave the analytical sample: mp 212–214 °C; $[\alpha]^{20}_D$ +28° (*c* 1, 95% EtOH). Anal. ($C_{22}H_{34}O_6$) C, H.

7β-Methyl-1,4-dibromo-1,4-seco-2,3-dinor-5α-androstan-17β-ol Acetate (4). A suspension of 1.74 g (4.4 mmol) of **3** and 1.45 g (6.7 mmol) of red HgO in 100 mL of CCl_4 was shielded from light and heated at reflux temperature with stirring. To the mixture there was slowly added 0.5 mL of Br_2 (ca. 9 mmol), and the reaction mixture was heated at reflux temperature for 3 h. After cooling, the mixture was filtered and the filtrate was evaporated under reduced pressure to give an oily residue. After purification with column chromatography on silica gel, using 3–4% acetone in petroleum ether (30–60 °C) as eluent, 1.5 g (73%) of the product **4** was obtained, mp 120–123 °C. Crystallization from 95% EtOH afforded the analytical sample: mp 123–125 °C; $[\alpha]^{20}_D$ +0.7° (*c* 1, $CHCl_3$). Anal. ($C_{20}H_{32}Br_2O_2$) C, H, Br.

7β-Methyl-2-thia-*A*-nor-5α-androstan-17β-ol (5). To a refluxing solution of 0.138 g (0.3 mmol) of **4** in 10 mL of ethyl alcohol there was added a tenfold excess of $Na_2S{\cdot}9H_2O$ in the least amount of hot water. The mixture was heated at reflux temperature for 18 h when TLC indicated the complete conversion of the dibromide to the product **5**. The solvent was evaporated under reduced pressure, and the solid residue was taken up in ether, washed several times with water, dried, and evaporated to give 0.077 g (87%) of white powder, mp 150–155 °C. Crystallization from hexane afforded the analytical sample: mp 163–164 °C; $[\alpha]^{20}_D$ +84° (*c* 1, $CHCl_3$). Anal. ($C_{18}H_{30}OS$) C, H, S.

7β-Methyl-2-thia-*A*-nor-5α-androstan-17β-ol Acetate (6). A solution of 0.70 g (2.38 mmol) of **5** in 10 mL of pyridine and 0.7 mL of acetic anhydride was allowed to stand at room temperature for 44 h. The mixture was poured into water and the aqueous solution was extracted with ether. The combined extracts were washed with dilute HCl and water, dried, and evaporated. The oily residue was chromatographed on silica gel, using 3–4% acetone in petroleum ether (30–60 °C) as eluent, to give a white

Table I. Myotrophic-Androgenic Assay

compd (total dose, mg)	wt, mg[a] ventrol prostate	seminal vesicles	levator ani	body wt, g initial	final
castrate control	17.5 ± 0.41	13.6 ± 0.19	23.6 ± 1.08	54	88
testosterone propionate (3.0)	126.0 ± 5.4	65.9 ± 3.7	68.2 ± 2.4	56	98
5α-DHT (1.0)	94.6 ± 7.2	50.5 ± 3.9	56.7 ± 4.4	56	91
2 (3.0)	81.4 ± 3.6	45.8 ± 3.2	63.3 ± 1.8	55	90
6 (3.0)	18.6 ± 1.6	11.8 ± 0.4	25.7 ± 2.0	58	90
2-thia-*A*-nor-5α-androstan-17β-ol acetate (3.0)[b]	61.9 ± 6.04	48.6 ± 3.56	65.2 ± 3.45	55	95

[a] Means plus or minus standard error (average of five rats). [b] See ref 5.

Table II. Tumor Therapy Summary[a]

treatment[b]	dose level, (mg/kg)/day	no. of anim surv/treated	FBW/IBW[c]	organ wt/100 g of FBW − tumor wt: tumor wt, g	% control	FTS[d] (L + W/2 mm)	% control
sesame oil	0.5 mL	10/10	1.56	6.65 ± 1.91		29.6 ± 3.3	
2	10.0	4/4	1.58	5.58 ± 2.39	84[e]	26.6 ± 3.6	90[e]
6	10.0	5/5	1.50	5.64 ± 1.81	85[e]	25.3 ± 3.4	85[f]

treatment	ovarian wt, mg	% control	uterine wt, mg	% control	spleen wt, g	% control	adrenal wt, mg	% control	thymus wt, mg	% control
sesame oil	55.5 ± 7.1		122.5 ± 16.4		0.43 ± 0.14		39.9 ± 6.5		176.0 ± 61.8	
2	42.6 ± 6.8	77[f]	174.9 ± 46.8	143[f]	0.33 ± 0.05	77[e]	27.3 ± 5.9	68[f]	124.6 ± 42.9	71[e]
6	58.2 ± 11.2	105[e]	152.5 ± 51.4	124[e]	0.35 ± 0.06	81[e]	40.3 ± 4.8	101[e]	220.7 ± 42.3	125[e]

[a] See ref 13. [b] All animals treated intraperitoneally. Treatment initiated on day 1 for 21 consecutive days. [c] Final body weight/initial body weight ratio. [d] Final tumor size (length + width/2 mm). [e] $p > 0.01$. [f] $p < 0.01$.

Table III. Tumor Therapy Summary[a]

treatment[b]	dose level, (mg/kg)/day	no. of anim surv/treated	FBW/IBW[c]	organ wt/100 g of FBW − tumor wt: tumor wt, g	% control	ovarian wt, mg	% control
sesame oil	0.5 mL	10/10	1.62	10.30 ± 2.33		51.7 ± 9.3	
testosterone acetate	100	10/10	1.68	12.73 ± 3.33	123[e]	33.5 ± 3.9	65[h]
calusterone[d]	100	4/4	1.64	12.71 ± 2.20	123[e]	30.9 ± 4.8	60[h]
7β-methyltestosterone[d]	100	4/4	1.58	10.65 ± 2.91	103[e]	37.3 ± 3.1	72[f]
2	100	5/5	1.81	13.63 ± 3.41	132[f]	32.0 ± 2.7	62[h]
6	100	5/5	1.55	13.70 ± 2.73	133[f]	45.1 ± 6.4	87[e]

treatment	uterine wt, mg	% control	spleen wt, g	% control	adrenal wt, mg	% control
sesame oil	97.3 ± 17.7		0.40 ± 0.07		38.5 ± 6.8	
testosterone acetate	175.5 ± 33.8	179[h]	0.40 ± 0.11	100[e]	34.0 ± 4.4	88[e]
calusterone[d]	145.3 ± 31.8	148[h]	0.46 ± 0.14	115[e]	28.0 ± 4.8	73[f]
7β-methyltestosterone	197.7 ± 51.0	202[h]	0.43 ± 0.07	108[e]	27.2 ± 7.0	70[f]
2	147.7 ± 14.7	151[h]	0.43 ± 0.10	108[e]	26.2 ± 5.5	68[h]
6	125.5 ± 13.6	128[h]	0.58 ± 0.18	145[f]	33.7 ± 4.0	87[e]

treatment	thymus wt, mg	% control	pituitary wt, mg	% control
sesame oil	130.4 ± 36.7		7.7 ± 0.9	
testosterone acetate	40.2 ± 18.3	31[h]	5.5 ± 0.6	71[h]
calusterone[d]	74.7 ± 32.8	57[f]	6.2 ± 0.8	80[f]
7β-methyltestosterone	62.1 ± 17.2	47[h]	5.6 ± 1.1	73[h]
2	62.6 ± 17.8	48[h]	4.3 ± 0.2	56[h]
6	104.3 ± 34.5	79[e]	6.9 ± 0.8	90[e]

[a] See ref 13. [b] All animals treated intraperitoneally. Treatment initiated on day 1 for 21 consecutive days. [c] Final body weight/initial body weight ratio. [d] Provided by the Upjohn Co. as gifts. [e] $p > 0.05$. [f] $p < 0.05$. [g] $p > 0.01$. [h] $p < 0.01$.

crystalline product. It was crystallized from petroleum ether (30–60 °C) to afford 0.42 g (53%) of **6**, mp 75–77 °C. Further crystallization from ethyl alcohol gave the analytical sample: mp 77–79 °C; $[\alpha]^{20}_D$ +75° (*c* 1, $CHCl_3$). Anal. ($C_{20}H_{32}O_2S$) C, H, S.

Pharmacological Testing. Myotrophic–Androgenic Assay.[10,11] The test compounds in carboxymethylcellulose (CMC) solution were given by subcutaneous administration once daily for 7 days to castrate male rats 21 days of age at the start of the test. Autopsy was performed on the day following the last day of administration. A total dose of 3 mg per rat of testosterone propionate was used as a standard. The results of the test are shown in Table I.

Testing for Antitumor Activity.[14] The 13762 mammary adenocarcinoma, originally DMBA (7,12-dimethylbenzanthracene) induced, is 100% transplantable and lethal in syngeneic Fischer 344 strain female rats. On day 0, grafts of the 13762 mammary tumor were implanted, subcutaneously, right side, into 19 Fischer 344 strain female rats at approximately 40 days of age. Treatment was initiated on day 1 and continued daily for 20 consecutive days. Test compounds were formulated in sesame oil and administered intraperitoneally. The control group was administered only the sesame oil vehicle at 0.5 mL per day. Twenty-four hours after the last treatment all animals were sacrificed for the various end-point determinations: IBW (initial body weight), FBW (final

body weight minus tumor weight), TW (tumor weight), FTS (final tumor size), spleen weight, ovarian weight, uterine weight, adrenal weight, thymus weight, and pituitary weight. The results of the test are shown in Tables II and III.

Acknowledgment. This investigation was supported in part by a Public Health Service Research Grant (AM-05016) from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service, and by funds from the Academic Senate, University of California, San Francisco, Calif. The testing for antitumor activity, performed without charge at Mason Research Institute, Worcester, Mass., is acknowledged with gratitude.

References and Notes

(1) H. Seidman, *Cancer*, **24**, 1355 (1969).
(2) J. A. Montgomery, T. P. Johnston, and Y. F. Shealy, in "Burger's Medicinal Chemistry", Part II, 4th ed, M. E. Wolff, Ed., Wiley-Interscience, New York, 1979, pp 637–640.
(3) T. L. Dao, in "Antineoplastic and Immuno-suppressive Agents", Part II, A. C. Sartorelli and D. G. Johns, Eds., Springer-Verlag, Heidelberg, 1975, Chapter 40.
(4) G. S. Gordan, A. Halden, Y. Horn, J. J. Fuery, R. J. Parsons, and R. M. Walter, *Oncology*, **28**, 138 (1973).
(5) M. E. Wolff, G. Zanati, G. Shanmugasundarum, S. Gupte, and G. Aadahl, *J. Med. Chem.*, **13**, 531 (1970).
(6) J. A. Zderic, H. Carpio, and H. J. Ringold, *J. Am. Chem. Soc.*, **81**, 432 (1959).
(7) R. E. Beyler, A. E. Oberster, F. Hoffman, and L. H. Sarett, *J. Am. Chem. Soc.*, **82**, 170 (1960).
(8) S. J. Cristol and W. C. Firth, Jr., *J. Org. Chem.*, **26**, 280 (1961).
(9) J. A. Davis, J. Herynk, S. Carroll, J. Bunds, and D. Johnson, *J. Org. Chem.*, **30**, 415 (1965).
(10) Pharmacological tests were performed at The Endocrine Laboratories, Madison, Wis.
(11) L. G. Hershberger, E. G. Shipley, and R. K. Meyer, *Proc. Soc. Exp. Biol. Med.*, **83**, 175 (1953).
(12) J. A. Vida, "Androgens and Anabolic Agents: Chemistry and Pharmacology", Academic Press, New York, 1969, pp 61–62 and 73–74.
(13) G. S. Gordan, A. Halden, and R. M. Walter, *Calif. Med.*, **113**, 1 (1970).
(14) Testing for antitumor activity was performed at the Mason Research Institute, Worcester, Mass.
(15) G. S. Gordan, private communication.
(16) Melting points were determined with a Thomas-Hoover apparatus equipped with a corrected thermometer. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. ORD–CD measurements and optical rotations were made with a Jasco ORD/UV-5 apparatus.

In Vivo Inhibitors of *Escherichia coli* Phenylalanyl-tRNA Synthetase

Daniel V. Santi,* Stephen O. Cunnion, Robert T. Anderson, and Robert W. Webster, Jr.

Department of Pharmaceutical Chemistry and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143. *Received May 3, 1979*

N-Benzyl-D-amphetamine is a potent in vitro and in vivo inhibitor of phenylalanyl-tRNA synthetase of *Escherichia coli*. The concentration of this inhibitor necessary for the in vivo inhibition is approximately 100-fold greater than that necessary for inhibition of the purified enzyme. Treatment of *rel*$^+$ strains of *E. coli* with the inhibitor results in a decreased percentage of $tRNA^{Phe}$ which is charged, guanosine tetraphosphate formation, cessation of RNA synthesis, and growth arrest. Evidence is presented which demonstrates that the primary and perhaps sole mode of action of *N*-benzyl-D-amphetamine is inhibition of phenylalanyl-tRNA synthetase.

In addition to the direct role of aminoacyl-tRNA's in protein biosynthesis, the charged/uncharged tRNA ratios function as regulators of growth and gene expression in prokaryotes and probably in eukaryotes.[1-3] Specific inhibitors of the aminoacyl-tRNA synthetases would be most useful tools for studying and manipulating the varied effects which might accompany depletion of intracellular charged tRNAs and should show profound effects on cell growth. While numerous inhibitors of cell-free preparations of these enzymes have been developed, relatively few have been convincingly demonstrated to be effective in vivo. The most studied of such inhibitors have been L-histidinol and *O*-methylthreonine, competitive inhibitors of His- and Ile-tRNA synthetases, respectively; while it is not clear that inhibition of the synthetases is the sole in vivo action of these analogues, it has been established that they do decrease the intracellular levels of charged $tRNA^{His}$ and $tRNA^{Ile}$.[3,4]

Recently, we have found that analogues of *N*-benzyl-2-phenylethylamine are extremely potent inhibitors of the Phe-tRNA synthetase from *E. coli*.[5] In this report, we describe experiments which demonstrate that this class of inhibitors is effective in vivo; one analogue, *N*-benzyl-D-amphetamine, has been studied in detail and demonstrated to specifically inhibit in vivo charging of $tRNA^{Phe}$ and produce the manifestations of the stringent response in *rel*$^+$ strains of *E. coli*.

Experimental Section

Carrier-free [^{32}P]phosphoric acid, [5-^{3}H]uracil (1.43 Ci/mmol), L-[^{3}H]phenylalanine (9.1 Ci/mmol), L-[^{3}H]isoleucine (105 Ci/mmol), and L-[^{3}H]valine (2.14 Ci/mmol) were products of New England Nuclear Corp. Polyethyleniminecellulose thin-layer sheets were obtained from Brinkmann Instruments and casamino acid was a product of Difco. The *N*-benzyl-2-phenylethylamine derivatives assayed for bacterial growth inhibition have been previously described.[5] Partially purified preparations of Phe-, Ile- and Val-tRNA synthetases were obtained from *E. coli* B (General Biochemicals) by published procedures[6-8] and were approximately 50% pure with respect to published values of the maximal rate of ATP–PP_i exchange.

Escherichia coli B/r, donated by N. Lee, is prototrophic and phenotypically stringent. Growth inhibition and reversal experiments with *E. coli* B/r were conducted at 37 °C in a basal salts medium containing 0.2% glucose, 0.7% K_2HPO_4, 0.3% KH_2PO_4, 0.01% $MgSO_4 \cdot 7H_2O$, and 0.1% $(NH_4)_2SO_4$. The medium used for all other experiments with *E. coli* B/r was the Tris–glucose minimal medium of Gallant and Suskind,[9] with or without

0022-2623/79/1822-1260$01.00/0