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5-Carboxamido-4-amino-3-isoxazolidone, an Asparagine Analogue

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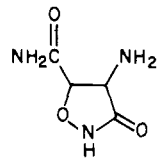
trans-Aziridine-2,3-dicarboxylic ester was used to prepare the required β-chlorohydroxamic acid used in the synthesis of the title compound. The trans configuration of the asparagine analogue was established by hydrogenolysis to *erythro*-β-hydroxyasparagine amide. Neither the title compound nor the intermediate aziridinehydroxamic acid (8) showed significant activity against the L1210 and P-388 tumors. The title compound was inactive as an inhibitor of asparagine synthetase from Novikoff hepatoma and did not inhibit the growth of some 25 bacteria and fungi.

The structural analogy among the title compound **1**,



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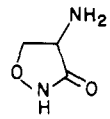
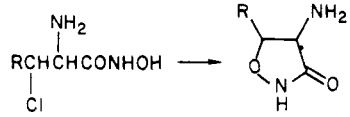


2

asparagine, and cycloserine (**2**) led us to prepare **1** and to investigate its antibacterial and anticancer activities.

The key intermediates in the synthesis of 5-substituted 3-isoxazolidones (**4**) are β-chlorohydroxamic acids such as

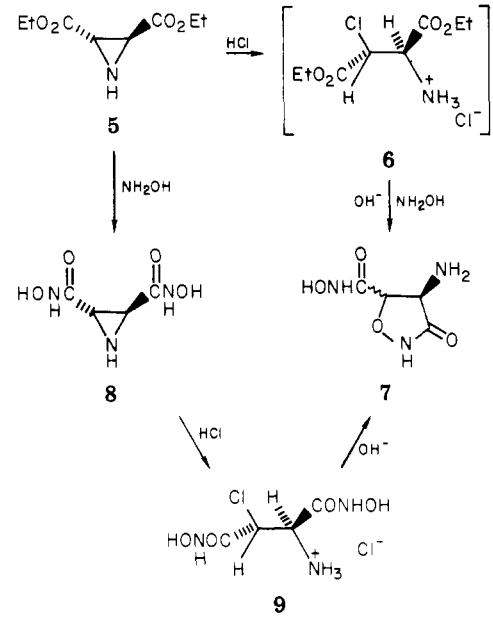


3 **4**

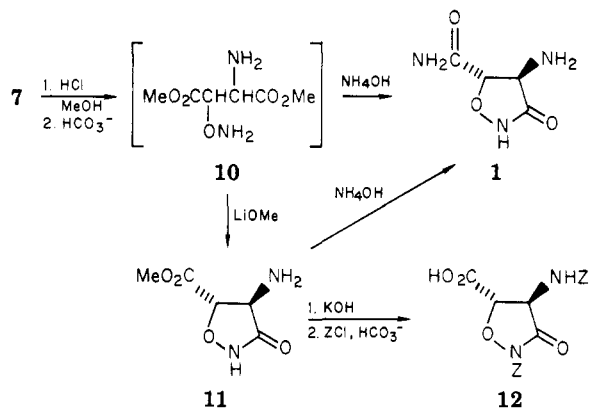
3 which can be cyclized[1-3] readily under basic conditions. Chloro compounds of this type have been previously obtained from oxazolines,[1] β-hydroxyamino acids,[2] and aziridines.[3] In our work toward **1**, we examined both a route using β-hydroxyaspartic acid and an oxazoline-4,5-dicarboxylic ester[4] as approaches to **3** without success. Since the aziridine-2,3-dicarboxylic ester **5** required in the Smrt[3] synthesis has recently been reported,[5] albeit without experimental details, this approach was examined more recently. Scheme I outlines the reaction sequence which yielded the desired product **1**.

The crystalline *trans*-aziridine (**5**) was obtained in 18% yield (from diethyl maleate) by the procedure of Berlin[5] et al., who obtained it as an oil having spectroscopic properties similar to our pure material. This intermediate was converted into the hydroxamic acid **7** by two methods. Excess hydroxylamine in the presence of methoxide reacted with **5** to form the dihydroxamic acid **8** in 83% yield. Dry hydrogen chloride in dimethoxyethane (DME) converted **8** into a chloro compound **9** which crystallized as a DME complex and was characterized spectroscopically. Treatment of **9** with aqueous base gave the cyclic hydroxamic acid **7**. This substance, probably a mixture of stereoisomers, was an amorphous solid which showed positive ninhydrin and ferric chloride tests and, more importantly, a positive nitroprusside test which is specific[6] for the isoxazolidone ring. Alternatively, the aziridine **5** could be converted as shown in Scheme I, without isolation of the intermediate oily chloro compound **6**, directly into **7**. The overall yields of **7** by the two processes were comparable and we felt that the route through **8** and **9**

Scheme I



Scheme II



served to confirm the structure of **7** since we were unable to get satisfactory elemental analyses for it.

The hydroxamic acid **7** readily underwent methanolysis (Scheme II) giving an equimolar mixture of the aminooxy diester **10** dihydrochloride and hydroxylamine hydrochloride. A cold aqueous solution of this mixture was neutralized and **10** was quickly extracted into chloroform. The oily **10** could then be converted directly into the desired amide **1** in approximately 50% yield by treatment with concentrated ammonium hydroxide. Complete

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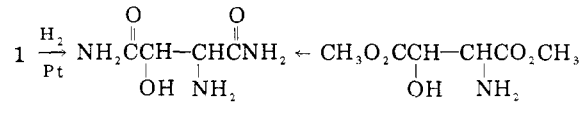
spectral and elemental analysis confirmed the structure of 1 and the stereochemistry was assigned as described below.

Since the starting aziridine **5** was of known configuration,[5] the reaction sequence should have produced a single isomer of 1 since each step is expected to be stereospecific. The fact that hydrogen chloride opening of the aziridine ring is known to be trans stereospecific and that the isoxazolidone ring closure most probably occurred by inversion of the β-carbon atom requires a cis configuration of the amino and carbohydroxamido groups in **7**. No further configurational changes should occur in the conversion of **7** to 1, so that the latter should also have the cis configuration. NMR spectroscopy was of no value in the determination of the configuration of **1** since an unresolved multiplet represented the C-4 and C-5 protons. In order to prove the configuration of 1, the ring was cleaved by hydrogenolysis, giving an 88% yield of the known[7] *erythro*-β-hydroxyasparagine amide, showing that the configuration was actually trans. For comparison, authentic samples of *threo*- and *erythro*-**13** were prepared



13 **14**

from the authentic dimethyl esters **14** by ammonolysis. Since the isoxazolidone ring is subjected to strongly basic conditions during the synthesis of **7** and again in the last step during ammonolysis, isomerization to the more stable trans configuration had apparently occurred due to the acidity of the C-5 proton which is adjacent to the carbomethoxy group. We believe the crude **1** to be a mixture of isomers with the trans compound predominating. The hydrogenolysis of **1** to **13** not only indicated its configuration but also confirmed the isoxazolidone structure.

It was also found that crude **10** could be converted into the isoxazolidone ester **11** in methanolic lithium methoxide. This ester gave **1** on treatment with ammonium hydroxide and could be hydrolyzed to the corresponding acid in potassium hydroxide solution. In spite of considerable effort, we were unable to free the acid completely of inorganic salts, an experience which we also had with the carbamyl derivatives[8] of cycloserine some years ago. Direct carbobenzyloxylation of the saponification mixture gave the bis derivative **12**, but again the acid was difficult to obtain free of salts. Finally, the use of cold aqueous trifluoroacetic acid allowed the isolation of an analytical sample of **12**. Strong acidification at higher temperatures led to ring hydrolysis and a mixture of acids. Synthesis of the bis(carbobenzyloxy) derivative **12** again confirmed the structure of 1.

Biological Evaluation. Both compounds 1 and 8 were assayed against L1210 leukemia in BDF_1 mice using a protocol which consisted of administration of 10^6 L1210 cells intraperitoneally on day 0, followed by an intraperitoneal dose of 100 mg/kg on day 1 only. Compound 8 conferred a 13% increase in life span while 1 conferred none. Compounds 1 and 8 were also assayed against P-388 tumor in mice using three schedules: (a) 200, 400, and 600 mg/kg, day 1 only; (b) 100, 200, and 400 mg/kg, days 1, 5, 9; (c) 25, 50, 100, and 200 mg/kg, days 1–9. A maximum antitumor effect of T/C 128% was noted for 8 at the 200 mg/kg dose level on the schedule c (days 1–9) regimen, while compound 1 showed no activity. Both compounds appear to be nontoxic to the host animals since all animals receiving the drugs survived the test period and showed minimal weight loss.

The asparagine analogue 1 was also tested[9] as an inhibitor of asparagine synthetase from Novikoff hepatoma. The testing was done in triplicate using freshly prepared 2 mM aqueous solutions of 1 to give 7, 0, and 0% inhibition as compared to 68, 64, and 62% inhibition by 2 mM solutions of N^{β}-(β-ethylfumaroyl)-α,β-diaminopropionic acid, a known[9] inhibitor. No inhibition by 1 was apparent.

It was also assayed in vitro against a broad spectrum of bacteria and fungi and showed no significant activity.[10]

Conclusion

The above results seem to indicate that although the aminoisoxazolidone ring system of cycloserine (**2**) can mimic D-alanine and consequently inhibit bacterial cell wall synthesis, the 5-carboxamido analogue 1 is unable to mimic asparagine in the systems investigated.

Experimental Section

All TLC determinations were carried out on Eastman Chromatogram Sheets No. 13254 cellulose with fluorescent indicator. Two solvent systems, butanol–acetic acid–water (3:1:1, BAW) and butanol–acetic acid–water (4:1:5, upper phase, BAWU), were used. Visualization was accomplished using ninhydrin (N), 1% $FeCl_3$ (F), and nitroprusside (NP) sprays. The NP test was carried out as follows. (1) Equal volumes of 4 N NaOH and 4% $K_3Fe(CN)_5NO$ were combined and sprayed on a sheet to give a yellow color, (2) dried at room temperature, and (3) sprayed with 50% aqueous HOAc until colorless; blue spots which fade in 5–15 min constitute a positive test. Where no TLC is reported, streaking occurred. For comparison, cycloserine showed R_f 0.48 (BAW), 0.54 (BAWU), N, NP.

***trans*-Diethyl Aziridine-2,3-dicarboxylate (5).** *rac*-Diethyl 2,3-dibromosuccinate prepared from 0.8 mol of diethyl maleate was added slowly with swirling to 700 mL of absolute EtOH saturated with ammonia in an ice bath. The reaction is exothermic in the first stage and the highest temperature depends on the scale of the reaction; the temperature should be maintained between 60 and 70 °C by external cooling but not below 60 °C. The mixture was left standing at room temperature for 2 h and evaporated in vacuo. Benzene was added to the residue, the insoluble material was filtered and washed with benzene, and the filtrate and washings were combined and evaporated in vacuo giving a yellowish oil. The oil was dissolved in 500 mL of hexane and chromatographed on a 600-g silica gel (200 mesh) column. Elution with benzene–hexane (1:1) gave 73.4 g of diethyl 2-aminofumerate as a colorless oil which, followed by elution with benzene–ether (5:1), gave a yellow oil which was crystallized from petroleum ether (bp 35–50 °C). Recrystallization from petroleum ether gave yellow needles of **5**: mp 51–54 °C; 26.6 g (18% from diethyl maleate). A small portion of the crystals was treated with alumina in petroleum ether and recrystallized from the same solvent to give colorless needles: mp 54–55 °C; IR (CCl_4) 3280 (NH), 1750, 1730 cm^{-1} (C=O); ^1H NMR (CCl_4) δ 1.30 (6 H, t, $-OCH_2CH_3$), 1.73 (1 H, d, J = 9 Hz, NH), 2.75 (2 H, d, J = 9 Hz, CH), 4.26 (4 H, q, OCH_2CH_3). Anal. ($C_8H_{13}NO_4$) C, H, N.

Diethyl β-Chloroaspartate Hydrochloride (6). A solution of **5** (14.96 g, 80 mmol) in 200 mL of CH_2Cl_2 was saturated with dry HCl at room temperature and allowed to stand at room temperature 1 h. The reaction mixture was evaporated in vacuo to give the oily chloro compound **6**: IR (neat) 3300–2300 (NH), 1750 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.16–1.43 (2 t, 6 H, $2\text{-}CH_2CH_3$), 4.43 (q, 4 H, $2\text{-}CH_2CH_3$), 5.38 (d, 1 H, J = 2 Hz, –CH–), 5.68 (d, 1 H, J = 2 Hz, –CH–), 9.26 (br, 3 H, $-^+NH_3$).

***trans*-Aziridine-2,3-dihydroxamic Acid (8).** Hydroxylamine hydrochloride (5.0 g, 72 mmol) and two drops of phenolphthalein solution were dissolved in 70 mL of hot, absolute EtOH, and the solution was neutralized with NaOEt–EtOH solution (prepared from 1.7 g of Na and 70 mL of absolute EtOH). The mixture was cooled in an ice bath for 10 min and filtered. The ester **5** (5.61 g, 30 mmol) and a LiOMe solution prepared from 420 mg of Li and 70 mL of absolute MeOH were added to the mixture with stirring at room temperature. After 16 h the mixture was cooled in ice, acidified to pH 2.5 with concentrated HCl, and stirred at room temperature for 2 h. The precipitate was filtered, washed with absolute EtOH, and recrystallized from H_2O–EtOH to give

4.0 g (83%) of **8** as colorless leaves. An analytical sample was recrystallized from H_2O and dried at 60 °C overnight: mp 157 °C dec; IR (Nujol) 3280, 3230, 3200–2100, 1690 (shoulder), 1660 cm^{-1}; ^1H NMR (D_2O) δ 2.86 (s, CH–). Anal. ($C_4H_7N_3O_4$) C, H, N.

4-Amino-5-carbohydroxamido-3-isoxazolidone (7). (a) From 6. **6** (prepared from 14.96 g of **5**) and NH_2OH·HCl (13.34 g) were dissolved in 120 mL of cold H_2O. To the stirred solution was added LiOH·H_2O (21.5 g) at −7 to −10 °C portionwise over a period of 20 min. The mixture was stirred at −7 to −10 °C for 30 min and then at −5 to −3 °C for 15 min and acidified (pH 4.5–5.5) with concentrated HCl at 0 °C. The solution was evaporated to ca. 70 mL under high vacuum below 0 °C and poured into 500 mL of absolute EtOH with stirring. After cooling 1 h at 5 °C, the precipitate was filtered and washed with absolute EtOH and ether, successively. The solid was dissolved in a minimum amount of H_2O and the solution poured into 400 mL of absolute EtOH with stirring. The precipitate was filtered and dried over $CaCl_2$ at room temperature in vacuo giving 12.2 g (95%) of an amorphous powder: IR (Nujol) 3400–2300 (NH), 1670, 1600 cm^{-1} (C=O); R_f 0.24 (BAW), 0.28 (BAWU), N, NP.

(b) From 9. The chloro compound **9** (3 g) was dissolved in 30 mL of cold H_2O. To the stirred solution cooled in an ice bath, LiOH·H_2O (1.68 g) was added portionwise over a period of 15 min. The mixture was stirred for 30 min, acidified (pH 5.0–5.5) with concentrated HCl at ice bath temperature, and evaporated to ca. 5 mL under high vacuum below 0 °C. The solution was worked up similarly to the above giving 0.9 g of colorless amorphous **7**. The IR and TLC were essentially identical with that of **7** obtained in part a.

β-Chloroaspartodihydroxamic Acid (9). Dry 1,2-dimethoxyethane (70 mL) was saturated with HCl at room temperature. The aziridine **8** (1.61 g, 10 mmol) was added to the solution at room temperature and the mixture stirred for 2 h, during which time the solid dissolved. Dry ether (200 mL) was added to the solution, and the mixture was stirred at room temperature until the insoluble material had changed to fine crystals which were filtered, washed with dry ether, and dried at room temperature giving 3.30 g of colorless crystals (**9**·HCl·DME): mp 85–88 °C dec; IR (Nujol) 3300–2300, (NH/OH), 1730 cm^{-1} (C=O); ^1H NMR (TFA–D_2O, 5:1) δ 3.40 (s, 6 H, OCH_3), 3.64 (s, 4 H, $-CH_2CH_2-$), 5.05 (AB q, 2 H, –CHCH–).

***trans*-4-Amino-5-carboxamido-3-isoxazolidone (1). Preparation of Dimethyl β-Aminooxyaspartate (10).** A suspension of **7** (6 g) in 60 mL of absolute MeOH was saturated with dry HCl at ice–water cooling temperature and refluxed gently for 20 min. The reaction mixture was evaporated in vacuo to give a semisolid product which as dissolved in a small amount of ice–water, neutralized with $NaHCO_3$, and quickly extracted with $CHCl_3$ (150 mL × 3). The organic layer was dried over Na_2SO_4 for 5 min and evaporated at room temperature, giving 3.8–4.2 g (53–59%) of crude **10** as a yellowish oil which was used immediately in the next step.

(a) 1 from 10. Crude **10** (1.6 g) was dissolved in 15 mL of concentrated NH_4OH and left standing at room temperature for 1.5 h. The reaction mixture was evaporated to dryness in vacuo at room temperature. The semisolid obtained was dissolved in 3 mL of H_2O, acidified (pH 6) with AcOH, and diluted with 5 mL of absolute EtOH. After cooling in a refrigerator for 1 h the precipitate was filtered, washed with 95% EtOH, and dried, giving 750 mg of crude **1**. Recrystallization as in part b gave 580 mg (48%) of light brown crystals whose IR was identical with that of **1** obtained in part b.

(b) 1 from 11. **11** (80 mg) was dissolved in 1 mL of concentrated NH_4OH and left standing at room temperature for 20 min. The mixture was evaporated in vacuo at room temperature to give crystals. The crystals were collected by the aid of absolute EtOH, washed with absolute EtOH, and dried to give 67 mg (92%) of colorless crystals, mp 190 °C dec. Recrystallization from NH_4OH–HOAc/EtOH in a manner similar to **11** gave an analytical sample of **1**: mp ca. 200 °C dec; IR (Nujol) 3420, 3280 (NH), 1680, (C=O), 1620 cm^{-1} (C=O); R_f 0.27 (BAW), 0.31 (BAWU), N, NP. Anal. ($C_4H_7N_3O_4$) C, H, N.

***trans*-4-Amino-5-carbomethoxy-3-isoxazolidone (11).** The crude **10** (6.6 g) was dissolved in 30 mL of absolute MeOH and stirred in an ice bath. To the above solution was added a LiOMe–MeOH solution (210 mg of Li was dissolved in absolute MeOH to give 15 mL of the solution) dropwise until the pH of the mixture was ca. 11. The mixture was stirred in an ice bath for 5 min, during which time small portions of LiOMe solution were added to keep the pH ca. 11. The reaction mixture was acidified (pH 5) with glacial AcOH, diluted with 30 mL of ether, cooled in an ice bath for 30 min, and filtered, and the solid was washed with absolute EtOH and dried to afford 2.15 g (62%) of **11** as colorless crystals, mp 160 °C dec. Crude **11** (100 mg) was suspended in 1 mL of H_2O, a minimum amount of concentrated NH_4OH was added to dissolve the solid, and the solution was treated with Norit A. Acidification with AcOH and dilution with 1 mL of EtOH gave an analytical sample (70 mg) of **11**: mp 168–170 °C dec; IR (Nujol) 3200–2300 (NH), 1760 (C=O), 1660 cm^{-1} (C=O); R_f 0.49 (BAW), 0.59 (BAWU), N, NP. Anal. ($C_5H_8N_2O_4$) C, H, N.

Hydrogenolysis of 1. **1** (1 mmol, 145 mg) (thrice recrystallized from NH_4OH–HOAc–EtOH) was hydrogenated using 80 mg of PtO_2 in 5 mL of H_2O at room temperature under atmospheric pressure. The reaction was completed in 20 min, during which time 24 mL of H_2 was absorbed. The catalyst was removed by centrifugation, and the supernatant solution was evaporated in vacuo to dryness giving crystals. Recrystallization from H_2O–MeOH gave 130 mg (88%) of colorless prisms: mp 170 °C dec; IR (Nujol) 3230, 3290, 3200 (NH), 1670 cm^{-1} (C=O); ^1H NMR (Me_2SO-d_6) δ ca. 3 (3 H, br, NH_2/OH), 3.20 (1 H, d, J = 4.5 Hz, $-CHNH_2$), 3.76 (1 H, d, J = 4.5 Hz, *CH*OH), 6.8 (4 H, br, 2-$CONH_2$); TLC identical with *erythro*-**13**. Anal. ($C_4H_9N_3O_4$) C, H, N.

Synthesis of Authentic β-Hydroxyasparagine Amides (13). *erythro*-13. A solution of *erythro*-**14**[11] in 20 mL of absolute MeOH was saturated with NH_3 in an ice bath and left standing at room temperature for 3 days. The precipitate formed was filtered, washed with MeOH, and recrystallized from H_2O–MeOH, giving 490 mg (67%) of colorless prisms: mp 166–167 °C dec; TLC R_f 0.50 (same system as *threo*-**13**), N. Recrystallization again from H_2O–MeOH gave a purified sample: mp 173 °C dec; IR and ^1H NMR were identical with the sample obtained by hydrogenolysis of **1**.

***threo*-13.** This compound was prepared from 1.06 g of *threo*-**14**[11] by the same procedure used for *erythro*-**13** in 72% yield: mp 189–193 °C dec; IR (Nujol) 3420, 3280, 3180 (NH/OH), 1690, 1660 cm^{-1} (C=O); ^1H NMR (Me_2SO-d_6) δ 2.8 (3 H, br, NH_2/OH), 3.23 (1 H, d, J = 2 Hz, *CH*NH_2), 4.05 (1 H, d, J = 2 Hz, *CH*OH), 6.8 (4 H, br, 2-$CONH_2$); TLC R_f 0.63 [EtOH–H_2O (7.3), Eastman chromatogram sheet 13181, silica gel], N. A sample of *threo*-**13** was dissolved in 1 N HCl and evaporated in vacuo at room temperature to give the hydrochloride. Recrystallization from MeOH gave pure sample: mp 213–215 °C dec (lit.[7] mp 190–192 °C dec); IR (Nujol) 3300, 3180 (NH), 1670 cm^{-1} (C=O). Anal. ($C_4H_{10}N_3O_3Cl$) C, H, N.

***trans*-2-Carbobenzyloxy-4-carbobenzyloxamido-5-carboxy-3-isoxazolidone (12).** The ester **11** (320 mg, 2 mmol) was stirred in 5 mL of 1 N KOH in an ice bath for 5 min. Small pieces of dry ice were added to the mixture to change the pH to ca. 8. Carbobenzyloxy chloride (850 mg, 5 mmol) and $NaHCO_3$ (252 mg) were added to the above solution, and the mixture was stirred for 1.5 h at room temperature. The reaction mixture was acidified to pH 4 with concentrated HCl and stirred with 5 mL of ether until fine crystals were formed. The crystals were filtered, washed with H_2O and ether successively, and dried to give 630 mg of product: mp 152–154 °C dec; IR (Nujol) 3340, 1790, 1750 (shoulder), 1730, 1700, 1550 cm^{-1}. This product was a mixture of the acid **12** and its sodium salt.

One drop of trifluoroacetic acid was added to a suspension of the salt (30 mg) in 1 mL of H_2O and the mixture was stirred at 0 °C for 10 min. The crystals were filtered, washed with H_2O, dried, and recrystallized from AcOEt–hexane to give 23 mg of **12**: mp 136–139 °C; IR (Nujol) 3360, 3200–3000, 1790, 1750, 1670 cm^{-1}; ^1H NMR δ [Me_2SO-d_6–$CDCl_3$ (1:3)] 4.9 (m, 2 H, CHCH), 5.25 (s, 2 H, CH_2Ph), 5.43 (s, 2 H, CH_2Ph), 5.8 (br, 1 H, NH), 7.5 (m, 10 H, C_6H_5). Anal. ($C_{20}H_{18}N_2O_8$) C, H, N.

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Steroid Antifertility Agents. Ionic Complexes of Basic Derivatives for Prolonged Action

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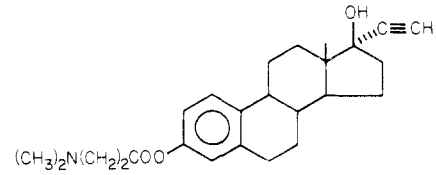
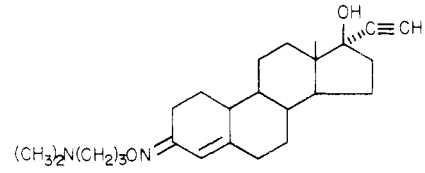
Ethynylestradiol 3-dimethylaminopropionate (**1**), norethindrone 3-(*O*-dimethylaminopropyl)oxime (syn and anti isomers, **2a** and **2b**), and testosterone 3-(*O*-dimethylaminopropyl)oxime (**3**) have been prepared and converted to zinc and aluminum tannate complexes as potentially long-acting prodrug forms of the parent steroids. The basic derivatives and the complexes showed the appropriate hormonal activities although they were less active in acute tests than the respective parents. The complexes of **1** showed prolonged activities and, in particular, the zinc tannate showed a prolonged duration of antifertility activity in the rat on subcutaneous administration in an aluminum monostearate gel.

In earlier reports we described the preparation of ionic complexes of narcotic antagonists with polyvalent metal ions and polybasic acids.[1-3] Several of these, notably naltrexone zinc tannate and naltrexone aluminum tannate, showed low aqueous solubility, a markedly prolonged duration of narcotic antagonist action, and a slowed rate of elimination after intramuscular injection in the form of fine suspensions in oil to mice,[1-4] rats,[4] guinea pigs,[4] and monkeys.[5] These results made it of interest, in light of the widely expressed need for long-acting antifertility agents, to investigate the feasibility of extrapolating our approach to the contraceptive steroids. Since to do this would require the use of steroids bearing basic substituents, we envisaged achieving our objective by attaching tertiary amino groups to steroids via biologically labile linkages. Conceptually then, our preparations would have a two-stage slow release process: (a) release of the base-substituted steroid from the complex; and (b) cleavage of the labile linkage and release of the pharmacologically active agent.

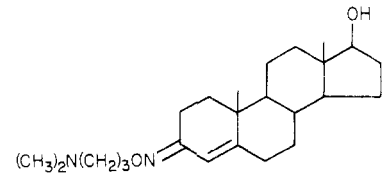
As a preliminary test of this strategem, we have synthesized basic derivatives of three representative steroids, specifically the estrogen ethynylestradiol, the progestin norethindrone, and the androgen testosterone. Derivatization involved attachment of a dimethylamino group via an ester or oximino function to provide ethynylestradiol 3-dimethylaminopropionate (**1**), norethindrone 3-(*O*-dimethylaminopropyl)oxime, syn and anti isomers (**2a** and **2b**), and testosterone 3-(*O*-dimethylaminopropyl)oxime (**3**). Both **1** and **2a** were found to have the appropriate hormonal activity (testing carried out by the National Institute of Child Health and Human Development) and were converted to zinc and aluminum tannate complexes.



1



2a, syn or anti
b, anti or syn



3

Prolongation of antifertility activity was demonstrated particularly with the zinc tannate complex of **1**.[6]

Considerable precedent exists for the use of ester derivatives of steroids as prodrugs subject to hydrolysis by plasma esterases to release the active principle.[7-9] Oxime derivatives of steroids have also shown hormonal activity. Oximes and O-substituted oximes derived from norethindrone have shown progestational activity comparable to

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