Separate samples were injected and measured either at 310 or 254 nm.

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Registry No. **3**, 68797-11-5; **4**, 23316-67-8; **5**, 38716-29-9; **6**, 89578-31-4; **7**, 68797-10-4; **8**, 89578-32-5; benzyl mercaptan, 100-53-8; 3-chloro-2-hydrazinopyrazine, 63286-28-2; *S*-adenosylhomocysteinase, 9025-54-1; adenosyl deaminase, 9026-93-1.

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Steroidogenesis Inhibitors. 1. Adrenal Inhibitory and Interceptive Activity of Trilostane and Related Compounds†

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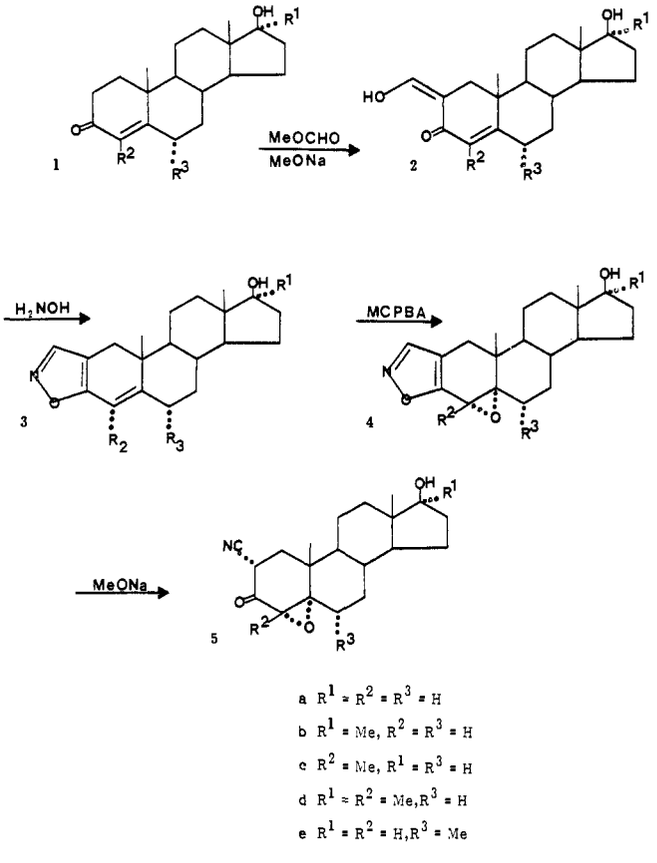
Several methylated derivatives of trilostane were prepared. Methylation of C-4 or C-4 and C-17 changes this relatively selective adrenal inhibitor to compounds with increased ovarian/placental inhibitory activity with decreased adrenal inhibitory activity.

Interceptives are agents that interrupt pregnancy after implantation,[1] a definition that qualifies trilostane (**5a**) as an interceptive agent in the rat and monkey,[2,3] The interceptive activity and adrenal inhibitory properties of trilostane result from inhibition of 3β-hydroxysteroid dehydrogenase.[2,3] An advantage of blockade at the 3β-hydroxy dehydrogenase step is that relatively innocuous precursors accumulate while inhibition at later stages of steroidogenesis results in the accumulation of or shunting to physiologically active intermediates. A further advantage of trilostane and its congeners is that they are devoid of hormonal or antihormonal activities.[2] At the clinical level, trilostane is effective in the treatment of primary aldosteronism,[4] reverses diuretic-induced hypokalemia,[5] decreases blood pressure in some patients with low renin hypertension,[6,7] and is useful in the treatment of Cushing's syndrome.[8] These results establish trilostane as an important therapeutic agent and confirm the revelance of the laboratory models used to develop the compound. Trilostane does not appear to quality as a useful interceptive agent, since much higher doses are required to terminate pregnancy in the rhesus monkey than are required to reduce adrenal steroidogenesis.[3]

This report and those to follow will relate the effect of molecular modification of trilostane on adrenal inhibitory activity and interceptive activity, with particular attention to (1) the identification of selective inhibitors of adrenal vs. ovarian/placental steroidogenesis and (2) the structural features required for carry-over of activity from the rat to the rhesus monkey. This report describes the synthesis and identification of compounds and compares the doses needed for interceptive activity with those that inhibit ACTH-stimulated glucocorticoid production.

Chemistry. The synthetic route to the three new methylated trilostane derivatives **5c–e** from the known testosterone derivatives **1c–e** is the same as that reported for trilostane (**5a**) and the 17-methyl analogue **5b**[9] (Scheme

Scheme I



a $R^1 = R^2 = R^3 = H$
b $R^1 = Me, R^2 = R^3 = H$
c $R^2 = Me, R^1 = R^3 = H$
d $R^1 = R^2 = Me, R^3 = H$
e $R^1 = R^2 = H, R^3 = Me$

Table I. Chemical Shifts of C-19 Protons at 100 MHz in Me_2SO-d_6

no.	δ, 19-H
5a	0.97
5e	0.97
14	1.06

† This paper was presented in part. See "Abstracts of Papers", 179th National Meeting of the American Chemical Society, Houston, TX, Mar 23–28, 1980; American Chemical Society, Washington, DC, 1981; Abstr MEDI 010.

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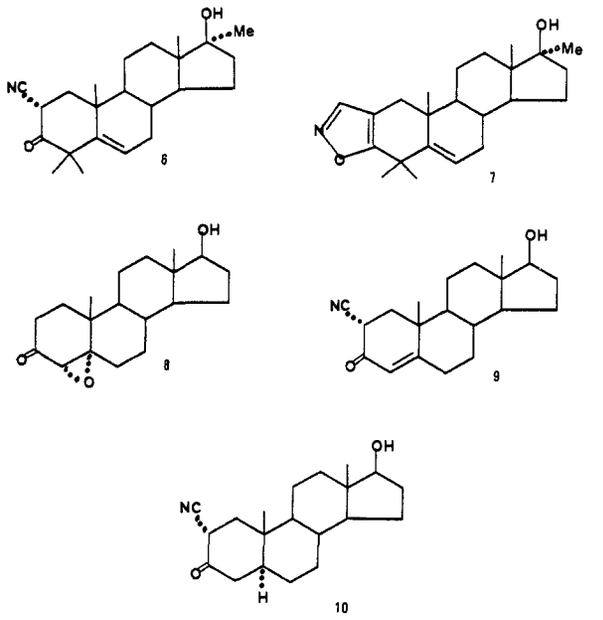
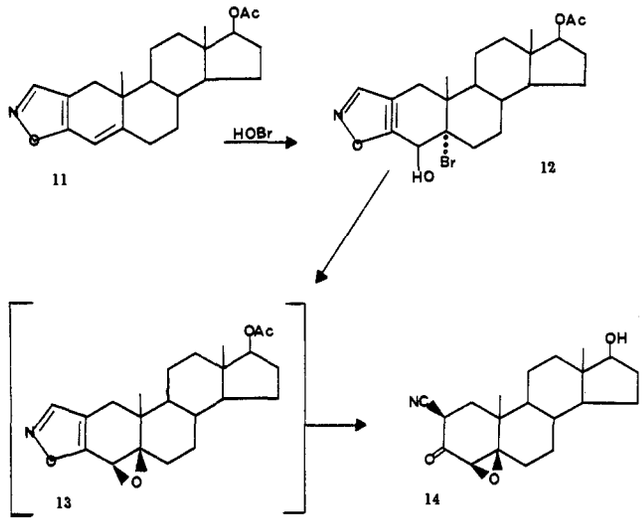
0022-2623/84/1827-0928$01.50/0

Chart I



Scheme II



I). Compounds **6** and **7** (Chart I) were prepared by the method of Manson et al.[10] Compound **8** was prepared by the method of Henbest et al.[11] Compound **9** was prepared by the method of Kissman et al.[12] Compound **10** was prepared by the method of Marchetti et al.[13] The synthesis of **14**, the 4β,5β-epoxy analogue of **5a**, is illustrated in Scheme II. The chemical shifts of the C-19 protons in the ^{1}H NMR spectra for the epoxides **5a**, **5e**, and **14** are given in Table I. These data show that the epoxide moiety in **5e** has the same configuration as in **5a** and is different from that in **14**. NMR and IR spectra of compounds **5a–e** in solution show that they are mixtures of keto–enol tautomers. For simplicity, in this paper they are shown only as their keto tautomers.

Table II. Interceptive Activity in the Rat and Monkey

no.	rat: MED_{10},[a,b] mg/kg po	monkey: MED_{90},[c] mg per monkey per day
5a	500	1000[d]
5b	500	250[d]
5c	125	>500[d]
5d	50	250[d]
5e	12	500[d]
4a	>500	not tested
4b	>500	not tested
4c	>500	not tested
4d	192	inactive (1000)[e]
4e	>500	not tested
6	6	inactive (1000)[f]
7	25	>1000[d]
8	inactive (500)	not tested
9	1000	not tested
10	inactive (1000)	not tested
14	inactive (1000)	not tested

[a] Minimum effective dose for 100% interruption of pregnancy when compound was administered once on the 10th day of pregnancy. [b] Seven rats per group. [c] Effective dose for interruption of pregnancy in greater than 90% of the monkeys when the compound was administered once each day on days 50–54 of pregnancy. [d] Six or more monkeys per group. [e] Two monkeys per group. [f] Three monkeys per group.

Table III. Effect of Compounds on Plasma Levels of Progesterone and Corticosterone in ACTH-Treated Pseudopregnant Rats

no.	MED[a] (progesterone)	MED[a] (corticosterone)
5a	500	50
5b	500	50
5c	5	50
5d	20	20

[a] Single dose, in milligrams per kilogram po, required to effect a significant ($p < 0.01$) decrease in hormone level as compared with stimulated control levels when administered to a group of seven rats.

Discussion

The interceptive activity of compounds **4–10** and **14** is presented in Table II. In the rat, the test compound was administered as a single oral dose on the 10th day of pregnancy. Activity in this test is considered to be a measure of the inhibition of both ovarian and placental function.[14] By contrast, compounds were tested in the monkey by administration on days 50 through 54. Activity observed in this test is a measure of inhibition of placental steroidogenesis.[15]

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Table IV. Effect on Plasma Levels of Cortisol in ACTH-Treated Rhesus Monkeys

no.	MED[a]
5a	10
5b	10
5c	250
5d	250

[a] Minimum single oral dose, in milligrams per monkey, required to effect a significant ($p < 0.01$) decrease in circulating cortisol levels when administered to a group of six monkeys.

In the rat, the interceptive activity of the trilostane derivatives was as follows: **5e > 5d > 5c > 5b = 5a**. In the monkey, **5b** and **5d** were more active than **5a** and **5c**; however, the 6α-methyl derivative **5e**, which was by far the most active of the five "epoxycyano ketones" in the rat, is undistinguished in the monkey.

Of the 4,5-epoxyisoxazole precursors, only **4d** exhibited appreciable activity in the rat. This compound, however, was not active in the monkey, even at a dose of 1000 mg.

Steroid **6**, the extensively investigated "cyano ketone"[16] is a noncompetitive inhibitor of 3β-hydroxysteroid dehydrogenase. It is very active in the rat, yet inexplicably inactive, even at a high dose, in the monkey. In contrast, the isoxazole **7**,[10] a competitive inhibitor of 3β-hydroxysteroid dehydrogenase, is active in the monkey but apparently inactive in the human.[17] The inactivity of **8**, **10**, and **14** and the lower order of activity in **9** in the rat show both the 2α-cyano group and the α-epoxide are necessary for optimal activity and appear to be necessary for carry-over of activity from the rat to the monkey. However, some of the differences in activity in the above species may relate to the observations noted above that interceptive activity in the rat[14] results from inhibition of ovarian and placental function, whereas activity observed in the monkey[15] interceptive test is the result of inhibition of the placental function alone.

Compounds **5a–d** were selected for an evaluation of their relative adrenal inhibitory and interceptive activities. In Table III are displayed the minimum effective doses required to effect a reduction of elevated progesterone and corticosterone levels in the ACTH-treated pseudopregnant rat. Compound **5e** was the only one of the four for which a significant decrease in progesterone levels occurred at a concentration well below that required to produce an effect on circulating elevated corticosterone levels. Compound **5a** lowered levels of both hormones at the same concentration and contrasts with **5a** and **5b** which were clearly far more inhibitory of adrenal function than of ovarian function in this test.

The doses of compounds **5a–d** that caused a significant decrease in circulating cortisol levels in the ACTH-stimulated monkey are shown in Table IV. A comparison of these figures with the minimum effective dose required to cause an interruption of greater than 90% pregnancy in the monkey (Table II) suggests that **5c** and **5d** interrupt pregnancy at concentrations that approximate those that inhibit steroid production from the hyperfunctional adrenal. With respect to the ratio of ovarian to adrenal inhibitory activity, **5c** and **5d** are clearly superior to **5a** and **5d**. Thus, methylation at C-4 or C-4 and C-17 of trilostane results in a decrease of adrenal inhibitory activity and an increase in interceptive activity in the monkey.

The results of a more detailed laboratory evaluation of **5d** have been reported elsewhere.[17,18] The evaluation of **5d** as an interceptive agent in humans is underway.

Table V. Physical and Analytical Data of New Compounds

no.	mp, °C	yield, %	recrystn solvent	formula[a]
3c	172–178	73	MeOH	$C_{21}H_{29}NO_2$
3d	193–194	74	Me_2CO	$C_{22}H_{31}NO_2$
3e	188–190	78	*i*-PrOAc	$C_{21}H_{31}NO_2$
4c	220–226	83	EtOH/EtOAc	$C_{22}H_{29}NO_2$
4d	242–252	87	THF/EtOH	$C_{22}H_{31}NO_3$
4e	220–222	83	EtOAc	$C_{21}H_{29}NO_3$
5c	192–193	79	EtOH/EtOAc	$C_{21}H_{29}NO_3$
5d	191–194	73	DMF/H_2O	$C_{22}H_{31}NO_3$
5e	247–249	67	Me_2CO/MeOH	$C_{21}H_{29}NO_3$

[a] Elemental analyses for C, H, and N were within 0.4% of the calculated value.

Experimental Section

Melting points were determined in capillary tubes and are uncorrected. MS, IR, ^1H NMR, and UV spectra of all compounds were consistent with their structures. Compounds **2–5** were prepared according to the previously described procedure.[9] Compounds **2c** and **2e** were used in the next step without purification. Compound **2d** was obtained as a solid (Et_2O), mp 197–204 °C, in 79% yield. Anal. ($C_{20}H_{32}O_3$) C, H.

(4β,5α,17β)-5-Bromoandrost-2-eno[2,3-*d*]isoxazole-4,17-diol 17-Acetate (12). To 500 mL of a dioxane solution of **11** (35.5 g, 0.1 mol) at 0–10 °C was added *N*-bromosuccinimide (35.5 g, 0.2 mol) in portions with stirring. After the final addition, the solution was clear, then became turbid, and then cleared up again with a transient red color over a 30-min period. TLC (ether) indicated mainly **11** plus a more polar spot. The flask was kept in the refrigerator overnight, after which **11** could no longer be detected and crystalline material had precipitated. The suspension was stirred in an ice bath, and saturated Na_2SO_3 solution was added in small portions until an aliquot gave a negative KI–starch paper test. The product was filtered and rinsed with H_2O, and the filtrate was neutralized with $NaHCO_3$. More precipitated product was collected. The combined crops were dried in vacuo at 60 °C, to give 40.25 g (89%) of a tan powder. The product was stirred in hot THF (150 mL), filtered, and washed (THF and EtOH) to give 30.6 g (68%) of **12**, mp 191–193 °C; NMR (Me_2SO-d_6) δ 1.10 (C-19 Me), 0.80 (C-18 Me), 5.05 (C-4 H). Anal. ($C_{22}H_{30}BrNO_4$) C, H, Br, N.

(2β,4β,5β,17β)-4,5-Epoxy-17-hydroxy-3-oxoandrostane-2-carbonitrile (14). In a nitrogen atmosphere at 20 °C, NaOMe (20 g, 270 mmol) was added to a stirred suspension of **12** (37.5 g, 83 mmol) in THF (1 L). The mixture, which gradually thickened, was stirred overnight. The THF was removed in vacuo, and the residue was dissolved in H_2O (1 L) and extracted with Et_2O to remove unreacted **12**. The aqueous solution was acidified with 2 N HCl to give 23.8 g (87%) of product. The analytical sample was obtained by recrystallization from DMF–H_2O: mp 258–261 °C; NMR (Me_2SO-d_6) δ 1.06 (C-19 Me), 3.20 (C-4 H); UV (95% EtOH) λ_{max} 253 nm (ε 8900), which should be compared with **5a**: NMR (Me_2SO-d_6) δ 0.97 (C-19 Me), 3.27 (C-4 H); UV (95% EtOH) λ_{max} 254.5 nm (ε 8090). Anal. ($C_{20}H_{27}NO_3$) C, H, N.

Biological Methods. The procedures for the interceptive and adrenal inhibitory tests in the rat and the monkey have been published.[2,3]

Note Added in Proof: In early human pregnancy **5d** has been shown to be a more selective inhibitor of progesterone secretion than **5a** and only interferes with adrenal steroid biosynthesis at high doses.[19]

Registry No. **1a**, 58-22-0; **1b**, 58-18-4; **1c**, 795-83-5; **1d**, 28626-76-8; **1e**, 13251-86-0; **2a**, 40996-87-0; **2b**, 10467-59-1; **2c**,

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71507-20-5; **2d**, 71507-12-5; **2e**, 38539-99-0; **3a**, 60413-79-8; **3b**, 13648-01-6; **3c**, 72166-52-0; **3d**, 71507-21-6; **3e**, 71507-13-6; **4a**, 20051-76-7; **4b**, 13647-38-6; **4c**, 71507-82-9; **4d**, 71507-78-3; **4e**, 71507-14-7; **5a**, 89999-20-2; **5b**, 13647-39-7; **5c**, 71507-83-0; **5d**, 71507-79-4; **5e**, 71507-15-8; **6**, 4248-66-2; **7**, 13074-00-5; **8**, 17597-25-0; **9**, 89999-17-7; **10**, 13648-06-1; **11**, 60413-80-1; **12**, 89999-18-8; **14**, 89999-19-9; progesterone, 57-83-0; corticosterone, 50-22-6; cortisol, 50-23-7.

Book Reviews

Chemical Information. By Yecheskel Wolman. Wiley, New York. 1983. xiv + 199 pp. 15.5 × 23.5 cm. ISBN 0-471-10319-5. $24.95.

The book bears the subtitle "A Practical Guide to Utilization" and was developed for a formal course in chemical literature at the Institute of Chemistry, Hebrew University of Jerusalem. It is printed in reduced typescript (with a sprinkling of typos) that does not allow use of bold face or italics. In an effort to crowd as much as possible into the text pages, lists of books, periodicals, and other types of literature are given in paragraph form rather than in tables. The book includes an index, a glossary of acronyms, and an appendix of 12 recent developments that are keyed to specific pages of the text.

The 14 chapters bear conventional headings, but they are not accurate descriptions of the chapter contents. Who would expect to find long discussions of "Bielstein", "Gmelin", and the "CA Subject Index" in a chapter on "Obtaining Numerical Data", especially when Beilstein was used as an alternative data source when a melting point couldn't be found in "Heilbron's Dictionary" or the "CRC Handbook"? There is a 4-page discussion on obtaining translations buried in a chapter on "The Library". The description of Landolt–Bornstein is found in a subsection dealing with crystallographic data. Phase diagram data sources are discussed under "Solubility Data". In an effort to avoid undue repetition, the author makes extensive use of a cross-reference system involving elaborately numbered paragraphs.

The unusual organization of the material is probably an expression of the author's lecture technique and also the result of his use of several specific search examples. The latter usually involve locating specific data, but there is one detailed description of a subject search (for Moebius dromaticity). The chapter on "How to Conduct a Literature Search" consists of just a 2-page exposition of 13 suggestions, the last of which is "know when to stop".

Among the deficiencies that must be a consequence of the book's brevity are inadequate coverage of the literature of chemical engineering, government publications, statistical data, chemical marketing, and biomedical properties. On the other hand, there are unusually complete discussions of current awareness techniques, spectral data, kinetics, the major treatises, and of Chemical Abstracts and its various indexes. The description of online searching is brief, but many references to extended discussions are given.

The literature of occupational hygiene, safety, toxicity, environmental impact, etc., is covered, but the author recommends that the ordinary chemist should not include safety literature in updating his reading program. He should rely on secondary sources even though they may be less or even more reliable than the original. There are so many such secondary sources today that one wonders where chemists can be found to prepare them. (Perhaps they use unemployed lawyers as authors.)

Dr. Wolman recommends that chemists today should devote 5–10 h per week in a personal current awareness program, supplemented by $500–1000 per year for computer service. An effective personal filing system should be developed. The use of a personal minicomputer is suggested to replace wives, now that the ladies no longer stay home to maintain the files.

The ACS Committee on Professional Training has recently completed a revision of the 1977 guidelines that emphasizes increased attention to information retrieval and states that students need formal instruction in this area. The book under review, along with 10 similar ones published during the past 10 years and with the help of commercially available tapes that teach the use of "Beilstein", "Gmelin", etc., collectively provide all that is needed for sound instruction in the use of the literature of chemistry. None of these texts addresses the critically important question of data quality; perhaps that can be learned only by bitter experience!

Amherst, Massachusetts **Edward R. Atkinson**

The Benzodiazepines: From Molecular Biology to Clinical Practice. Edited by Ermineo Costa. Raven Press, New York. 1983. xiv + 432 pp. 16 × 24 cm. ISBN 089004-885-I. $39.50.

In the period since the benzodiazepines were introduced into clinical practice over 20 years ago their popularity has increased until they have now become one of the most commonly prescribed classes of drugs in the world. The sheer volume of their usage combined with occasional reports of withdrawal symptoms or adverse reactions have, however, been reason for concern both by drug regulatory agencies and by the general public. This important topic of possible overuse or abuse of the benzodiazepines was a subject for thoughtful discussion by several authors in this compilation of papers presented at a World Congress of Biological Psychiatry symposium held in Stockholm in July 1981. It was further considered in a round table discussion by leading investigators from around the world and chaired by Dr. Leo Hollister.

Other clinical papers in this volume deal with the possible use of benzodiazepines for treating a variety of centrally mediated disorders, including schizophrenia, endogenous depression, and epileptic syndromes, in addition to their use for treating insomnia and anxiety. Their intravenous use in anesthesiology was also discussed. The safety of the benzodiazepines was considered from several points of view, including their interaction with other substances, particularly alcohol or other depressants, their metabolism, pharmacokinetics and use in patients with impaired liver or kidney function, their use in elderly patients, and their effect on performance, especially when used chronically for treating anxiety or insomnia.

As important as these topics are, especially for the practicing physician, in my opinion they are overshadowed by the remarkable strides that have been made on elucidating the mechanism by which the benzodiazepines interact with the central nervous system. These discoveries will undoubtedly have a tremendous impact on our understanding of central nervous system function and may lead to new approaches for therapeutic intervention in a variety of diseases. The "benzodiazepine receptor" is now considered to be an integral part of a GABA–receptor complex which also includes a GABA recognition site, a regulator protein (GABA modulin), and a chloride channel. It is believed that the "benzodiazepine receptor" is the recognition site for a co-transmitter that appears to decrease the action of GABA on the receptor. Interaction of the anxiolytic benzodiazepines with this recognition site increases the B_{max} for GABA binding and, thus, increases GABAergic transmission. It is possible that the insight gained through these investigations of the GABA receptor may be applicable to other transmitter systems. If so, it suggests that it might be possible to find useful chemical modulators for other transmitters or perhaps different, more selective modulators for the GABA system. This volume includes excellent discussions of the mechanism of benzodiazepine action, including discussions