Notes

Griseofulvin Analogs Derived from Transformations of 5′-Formylgriseofulvin[1]

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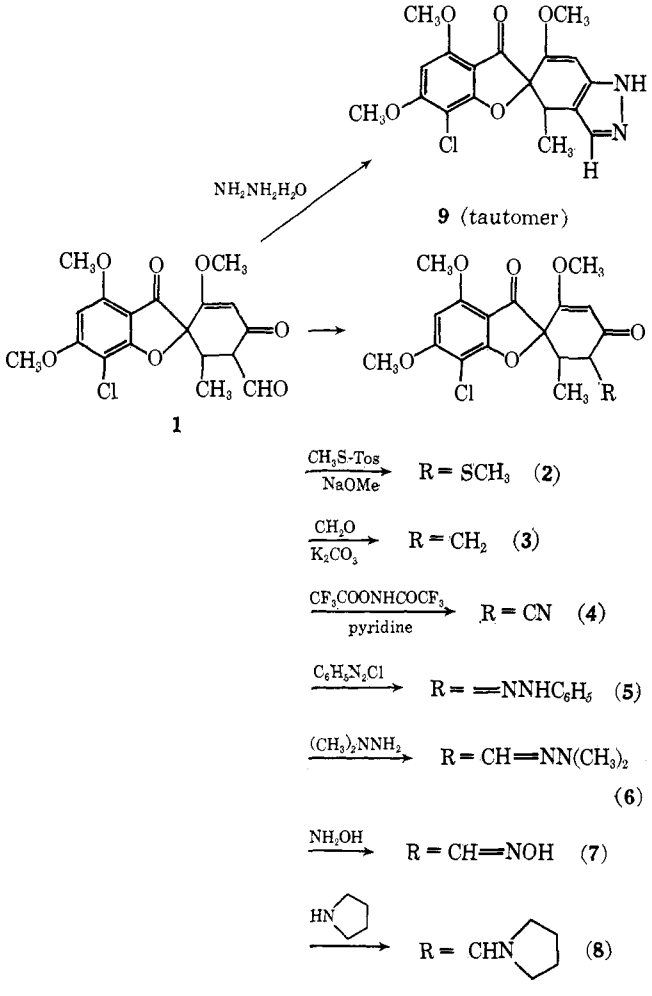
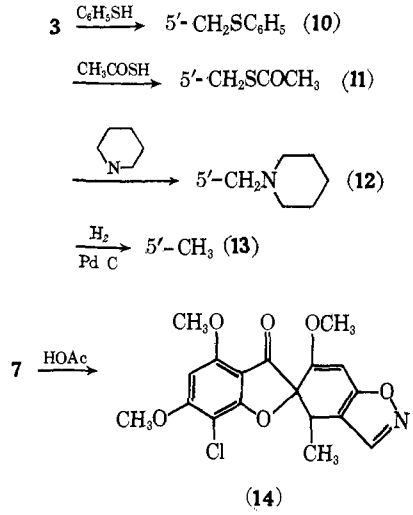
In our previous publication[2a] we described a simple high yield preparation of 5′-formylgriseofulvin (**1**) and its conversion to various halogen derivatives. We describe here its further transformation into a variety of 5′-griseofulvin analogs as outlined schematically in Scheme I.[2b]

SCHEME I



A few of these 5′-derivatives were further transformed as indicated in Scheme II.

SCHEME II



The transformations listed are all previously described reactions of β-formyl ketones and need no further elaboration here.

Biological Data.[3]—In Table I are listed the *in vitro* testing results of compounds **1–14**, along with some of those 5′-griseofulvin derivatives whose preparations have been reported previously.[2a]

As can be seen several of them are quite highly active. The 5′-ethoxalyl and 5′-methoxalyl analogs are about as active as griseofulvin while the isoxazole **14** appears to be even more active.

Compounds **1–14** with the exception of **12** were evaluated *in vivo* against *Microsporum canis* in guinea pigs topically in Carbowax at 1%. (Griseofulvin under these conditions is highly active). All except **7** were inactive. Although **7** did show some activity, it was considerably less active than griseofulvin.

Experimental Section[4]

5′-Methylthiogriseofulvin (2).[5]—5′-Formylgriseofulvin (381 mg, 1 mmole) was slurried in 6.5 ml of EtOH contg 54 mg (1 mmole) of NaOMe. The mixt was brought to reflux and methylthiotosylate (202 mg, 1 mmole in 4 ml of EtOH) was added dropwise over a 10-min period. The pale yellow soln was refluxed an additional 12 min. It was cooled in an ice bath, and the white crystals were collected by filtration, washed with H_2O, and dried *in vacuo;* yield, 256 mg (64.1%). An anal. sample was obtained by recrystn from MeCN, mp 241–242°. *Anal.* ($C_{18}H_{19}ClSO_6$): C, H, Cl, S.

5′-Methylenegriseofulvin (3).[6]—A soln of 381 mg (1 mmole) of 5′-formylgriseofulvin in 20 ml of Me_2CO was stirred vigorously with 1.0 g of freshly powd K_2CO_3 and 1.0 ml of 37% aq CH_2O

(1) Griseofulvin Analogs. 9; part 8, T. Fields, H. Newman, and R. B. Angier, *J. Med. Chem.*, **13**, 1242 (1970).

(2) (a) H. Newman and T. L. Fields, *J. Org. Chem.*, **35**, 3156 (1970); (b) the prepn of 5,-diazogriseofulvin from **1**, some of its transformation products, and their biological activities have also been described; T. Fields, H. Newman, and R. B. Angier, *J. Med. Chem.*, **13**, 1242 (1970).

(3) The *in vitro* and *in vivo* testing data were supplied by Mr. A. Dornbush and Mr. G. Redin and staffs, respectively, of the Chemotherapy Research Section of these laboratories.

(4) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Melting points are uncor. The nmr spectra were detd on a Varian A-60 spectrometer, ir spectra in KBr discs on a Perkin-Elmer Infracord spectrophotometer. The nmr and ir spectra were consistent with the assigned structures.

(5) The general procedure of R. L. Autrey and P. W. Scullard, *J. Amer. Chem. Soc.*, **90**, 4921 (1968), was followed.

(6) The general procedure of A. J. Manson and D. Wood, *J. Org. Chem.*, **32**, 3434 (1967), was followed.

TABLE I

In Vitro ANTIFUNGAL ACTIVITY DATA (AGAR DILUTION) MINIMAL INHIBITORY CONCENTRATIONS IN μg/ml[a,b]

Compd	Ca	Cn	Mc	Mg	Pj	Tt	Tm	Tr
1	—	—	5	50	—	25	25	10
2	—	—		25	—	25	25	10
3	—	—		25	—	25	10	10
4	—	—	2.5	5	—	10	10	5
5	—	—	62	250	—	—	—	250
6	—	—	25	25	—	50	50	25
7	—	—	5	10	—	25	25	10
8	—	—	10	25	—	25	50	25
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—
12	—	—	50	100	—	100	100	100
13	—	—		25	—	25	50	25
14	—	—	0.25	0.5	—	2.5	2.5	1
5′-Ethoxalyl	—	—	1	5	—	5	5	1
5′-Methoxalyl	—	—	0.5	2.5	—	5	5	1
5′-Chloro	—	—		10	—	10	25	25
5′-Bromo	—	—		10	—	10	25	25
5′-Fluoro	—	—	1	2.5	—	10	10	10
5′-Iodo	—	—		10	—	10	100	10
5′-Fluorogriseofulvic acid	—	—	—	—	—	—	—	—
Griseofulvin	—	—	1	1	—	10	2.5	1

[a] A solid dash denotes inactivity at 250 μg/ml; a blank indicates not tested. [b] The initials heading the columns represent the following fungi (from left to right): *Candida albicans, Cryptococcus neoformans, Microsporum canis, Microsporum gypseum, Phialophthora jeanselmei, Trichophyton tonsurans, Trichophyton mentagrophytes,* and *Trichophyton rubrum.*

at room temp for 40 min. The reaction mixt was then gradually dild with 100 ml of H_2O. The K_2CO_3 dissolved and then a white solid pptd. This was collected on a filter, washed with H_2O, and dried *in vacuo;* yield, 300 mg (82%). An anal. sample was obtained by recrystn from EtOH, mp 205–207°. *Anal.* ($C_{18}H_{17}ClO_6$): C, H, Cl.

5′-Cyanogriseofulvin (4).[7]—*O,N*-Bis(trifluoroacetyl)hydroxylamine (2.25 g, 10 mmoles) and pyridine (3 ml) were added to a slurry of 5′-formylgriseofulvin (3.81 g, 10 mmoles) in 50 ml of C_6H_6. The reaction mixt was brought to reflux and within 15 min a clear soln was obtained. Refluxing was continued for 3.5 hr. After cooling, the C_6H_6 soln was washed 3 times with H_2O, dried (Na_2SO_4), filtered, and concd to a yellow–tan solid *in vacuo*. The crude nitrile was slurried in 25 ml of EtOH at reflux temp and then cooled in an ice bath. The white crystals of anal. pure **4** were collected and dried *in vacuo* over P_2O_5 at 80°. The yield was 2.8 g (74.4%). *Anal.* ($C_{18}H_{16}NClO_6$): C, H, Cl, N.

5′-Oxogriseofulvin 5′-Phenylhydrazone (5).—A suspension of 5′-formylgriseofulvin (3.81 g, 10 mmoles) and NaOAc (5 g) was cooled to 5° in an ice bath. A soln of PhN_2Cl, obtained from 10 mmoles of $PhNH_2$, was added dropwise and the resultant bright yellow suspension was stirred at 5° for 40 min and at room temp for 1 hr. The yellow solid was collected by filtration, washed twice with H_2O, then with MeOH, and dried *in vacuo*. The yield of crude phenylhydrazone was 4.27 g (93%). An anal. sample was obtained by two recrystns from MeOH, mp 233–235°. *Anal.* ($C_{23}H_{21}ClN_2O_6$): H, Cl, N; C: calcd, 60.45; found, 60.95.

5′-Formylgriseofulvin *N,N*-Dimethylhydrazone (6).—Dimethylhydrazine (198 mg, 3.3 mmoles) was added dropwise to a suspension of 5′-formylgriseofulvin (1.143 g, 3 mmoles) in 10 ml of MeOH. The suspension was stirred at room temp for 1.5 hr and then refluxed for 0.5 hr. Upon refluxing, a clear yellow soln was obtained which deposited crystals upon cooling. After cooling in an ice bath the pale yellow crystals were collected and dried *in vacuo*, at 80°. The yield of **6** was 0.980 g (70%). An anal. sample was obtained by recrystn from EtOH. *Anal.* ($C_{20}H_{23}ClN_2O_6$): C, H, Cl, N.

(7) The general procedure of J. H. Pomeroy and C. A. Craig, *J. Amer. Chem. Soc.*, **81**, 6340 (1959), was followed.

5′-Formylgriseofulvin 5′-Oxime (7).—A mixt of 5′-formylgriseofulvin (4.58 g, 12 mmoles), $HONH_2 \cdot HCl$ (0.92 g, 13.2 mmoles), and NaOAc (1.08 g, 13.2 mmoles) in 70 ml of MeOH was stirred at room temp for 2 hr. The slurry was then poured into 400 ml of H_2O and the light tan solid was collected by filtration, washed well with H_2O, and dried *in vacuo*. The yield of crude oxime was 3.95 g (83%), mp 228–231°. An anal. sample was obtained by recrystn from MeCN, mp 240–242°. *Anal.* ($C_{18}H_{18}ClNO_7$): C, H, Cl, N.

5′-Pyrrolidinomethylenegriseofulvin (8).—A cooled (ice water) soln of 19 g (0.05 mole) of 5′-formylgriseofulvin in 100 ml of CH_2Cl was treated with 3.6 g (0.05 mole) of pyrrolidine and the mixt was stirred at room temp for 1.5 hr. The org phase was decanted from some insol oil which had formed and evapd to leave an orange foam which crystd on trituration with C_6H_6; yield, 20.5 g (95%), mp 149–153° (with shrinking 140–145°). *Anal.* ($C_{22}H_{24}NCl \cdot 0.5C_6H_6$): C, H, Cl, N. The presence of 0.5 mole of C_6H_6 was confirmed by nmr spectroscopy.

Spiro[benzofuran-2(3*H*),5′(5*H*)-indazole]-3-one-7-chloro-2′,4′-dihydro-4,6,6′-trimethoxy-4′-methyl(4′,5′-pyrazologriseofulvin) (9).—5′-Formylgriseofulvin (1.26 g, 3.3 mmoles) and hydrazine hydrate (150 mg, 3.0 mmoles) were slurried in 10 ml of MeOH at room temp. Within a few min, a clear, yellow soln formed. The mixt was refluxed for 45 min and then cooled in an ice bath. The small amount of solid which pptd was removed by filtration and discarded. The clear filtrate was dild with 50 ml of 0.5 *N* HCl, and the solid was filtered off and discarded. The filtrate was basified with 1 *N* NaOH. The white solid which pptd was collected on a filter, washed well with H_2O, and dried *in vacuo* at 80°. The yield of crude 4′,5′-pyrazologriseofulvin was 0.5 g (32%). A sample which analyzed satisfactorily for the desired compound containing 2 moles of AcOH was obtained by first crystg from MeOH and then from gl AcOH, mp softens 160°, liq 234°. The nmr spectrum was consistent with this formulation. *Anal.* ($C_{18}H_{17}ClN_2O_5 \cdot 2CH_3COOH$): C, H, Cl, N.

5′-Phenylthiomethylgriseofulvin (10).—5′-Methylenegriseofulvin (150 mg, 0.41 mmole) was slurried in 1.0 ml of thiophenol and the mixt was stirred at room temp for 3 hr. The clear soln was then partitioned between CH_2Cl_2 and 1 *N* NaOH. The org layer was washed with H_2O and after drying (Na_2SO_4), it was concd to a syrupy residue *in vacuo*. Crystn from 4 ml of EtOH gave 81 mg (42%) of **10**, mp 180–190°. Recrystn from EtOH raised the mp to 201–204°. *Anal.* ($C_{24}H_{23}ClSO_6$): H, Cl, S; C: calcd, 60.70; found, 60.23.

5′-Acetylthiomethylgriseofulvin (11).—5′-Methylenegriseofulvin (100 mg, 0.262 mmole) was dissolved in 2 ml of thiolacetic acid. The clear soln was stirred at room temp for 30 min and then refluxed for 30 min. After standing an addnl 2 hr at room temp, the reaction mixt was poured into 40 ml of H_2O and basified by the addition of 10 *N* NaOH. The basic mixt was extd 3 times with $CHCl_3$, and the combined exts were washed with H_2O, dried (Na_2SO_4), filtered, and concd *in vacuo*. The residual oily solid upon crystn from 5 ml of EtOH yielded 91 mg (79%) of pale yellow **11**, mp 210–212°. *Anal.* ($C_{20}H_{21}ClSO_7$): C, H, Cl, S.

5′-Piperidinomethylgriseofulvin (12).—A soln of 5′-methylenegriseofulvin (1.46 g, 4 mmoles) and piperidine (340 mg, 4 mmoles) in 25 ml of CH_2Cl_2 was stirred at room temp for 24 hr. The soln was extd 3 times with 15-ml portions of 1 *N* HCl. The combined exts were washed with Et_2O, cooled in an ice bath, and basified with 1 *N* NaOH. The white solid which pptd was collected by filtration, washed with H_2O, and dried *in vacuo* at 80°. The yield of anal. pure **12** was 214 mg, mp 165° (softens, 120°). *Anal.* ($C_{23}H_{28}ClNO_6$): C, H, Cl, N.

5′-Methylgriseofulvin (13).—A soln of 5′-methylenegriseofulvin (2.0 g, 5.5 mmoles) in 150 ml of 1,2-dimethoxyethane contg 1.0 g, of 10% Pd/C was subjected to an H_2 atmosphere at room temp and atm pressure. The theoretical amt of H_2 was taken up in 20 min and the reaction stopped. The catalyst was removed by filtration and the filtrate was concd to an off-white solid *in vacuo*. Recrystn from 10 ml of EtOH yielded 1.0 g (50%) of white cryst **13**. An anal. sample was obtained by recrystn from EtOH, mp 205–208°. *Anal.* ($C_{18}H_{19}ClO_6$): C, H, Cl.

Spiro[1,2-benzisoxazole-5(4*H*),2′(3′*H*)-benzofuran]-3′-one-7′-chloro-4′,6,6′-trimethoxy-4-methyl(4′,5′-isoxazologriseofulvin) (14).—A slurry of the oxime of 5′-formylgriseofulvin (100 mg) in 3 ml of gl AcOH was stirred at room temp for 45 min and then refluxed for 2 hr. After cooling to room temp, the slurry was poured into 40 ml of H_2, and the white solid was collected by filtration. The yield of crude isoxazole was 73.5 mg (75%), mp,

218–222°. An anal. sample was obtained by recrystn from MeOH, mp 228–229°. *Anal.* ($C_{18}H_{16}NClO_6$): C, H, Cl, N.

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Synthesis and Biological Response of Some 3-Iminoprogestins

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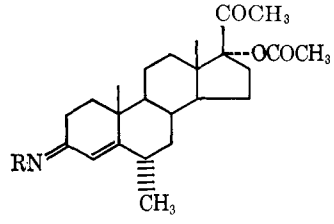
In a recent study[1] it was observed that the introduction of a 6α-Me group onto 17α-acetoxyprogesterone oxime enhanced the progestational response tenfold. At the same time introduction of a long-chain fatty ester at C-17 decreased the activity precipitously. The present study was initiated with 6α-methyl-17α-acetoxyprogesterone oxime to ascertain if varying the basicity at C-3 molecular modification would effect the progestational response.

The compounds listed in Table I were synthesized

TABLE I
3-OXIMINO ESTERS



Compound[a] No.	R	Recrystal[b] solvent	Mp, °C	Formula[c]
1	CH_3COO	M–W	104–107	$C_{26}H_{37}NO_5$
2	C_2H_5COO	M–W	81–85	$C_{27}H_{39}NO_5$
3	$CH_3[CH_2]_4COO$	M–W	105–108	$C_{30}H_{45}NO_5$
4	C_6H_5COO	M	190–193	$C_{31}H_{39}NO_5$
5	C_6H_5	H	133–135	$C_{30}H_{39}NO_3$
6	C_6H_5NH	M	154–157	$C_{30}H_{40}N_2O_3$
7	NH_2CONH	A	246–248	$C_{25}H_{37}N_3O_4$

[a] All compds showed uv absorption around 240 nm. [b] A = Me_2CO; H = hexane; M = MeOH; W = H_2O. [c] Acceptable C, H, N values were obtained for all compds.

according to the procedure outlined in the Experimental Section. The spectral data and elemental analyses confirm the structural assignments. These compounds were studied for their *in vivo* progestational response as well as their ability to bind *in vitro* to a specific progesterone receptor site.[2]

The data for the Clauberg test[3] are shown in Table II as the McPhail index.[4] It is apparent from the response of **1**, **2**, **3**, and **7** that decreasing the polarity of the parent molecule or increasing the basicity did not significantly alter the McPhail index. Interestingly

(1) A. P. Shroff, *J. Med. Chem.*, **13**, 748 (1970).
(2) J. L. McGuire and C. DeDella, *Endocrinology*, in press.
(3) C. Clauberg, *Zentralbl. Gynaekol.*, **54**, 2757 (1930).
(4) M. K. McPhail, *J. Physiol. (London)*, **83**, 145 (1935).

TABLE II
PROGESTATIONAL RESPONSE OF RABBIT UTERUS

Compound No.	Dose, mg/kg	McPhail index
1	0.5	3.2
2	0.5	2.9
3	0.5	3.1
4	1.0	3.3
5	1.0	3.2
6	1.0	3.2
7	0.5	3.45

4, **5**, and **6**, having a bulky Ph group in common, require twice the dose for similar response.

Binding studies of these compounds to uterine progestin receptor were carried out according to the method outlined in the Experimental Section. McGuire and DeDella[2] have published data suggesting the existence of a specific progestin receptor in the rabbit uterus. All of the compounds from Table I were studied for their ability to bind to this receptor by identical techniques. The binding response as tabulated in Table III rep-

TABLE III
UTERINE PROGESTIN RECEPTOR BINDING RESPONSE

Compound No.	Binding response[a]
1	+++
2	+++
3	+++
4	+++
5	+++
6	+++
7	+

[a] (+++) strong binder; (++) medium binder; (+) poor binder.

resents the displacement of progesterone-*t* from the receptor by the compound. It is evident that all compounds bind very strongly to the progesterone receptor site except **7**. The discrepancy between the *in vivo* Clauberg test for **7** and the *in vitro* receptor binding response may be due to metabolism of **7**, or the C-3 terminal amino group may offset the binding dimensions.

Experimental Section

All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. The uv and ir data were obtained on a Cary Model 11 and Beckmann IR-5 spectrophotometers, respectively. Microanalyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements (Table I), analytical results obtained for these elements were within ±0.4% of the theoretical value.

General Procedure for 1, 2, 3, and 4. 3,17α-Diacetoxy-6α-methylpregn-4-ene-3,20-dione 3-Oxime (1).—A soln contg 0.5 g of 17α-acetoxy-6α-methylpregn-4-ene-3,20-dione 3-oxime in 1.5 ml of Ac_2O and 0.7 ml of C_5H_5N was stirred for approx 10 min and poured over ice water. The crude crystals thus formed were collected by filtration and recrystd from MeOH–H_2O. The yield of 1, mp 104–107, was 88%.

3-Iminobenzene-17α-acetoxy-6α-methylpregn-4-en-20-one (5).—17α-Acetoxy-6α-methylprogesterone (1 g) was treated with 10 ml of $PhNH_2$ and refluxed for 24 hr. Excess aniline was removed under reduced pressure and the residue was extd with boiling hexane. On cooling the hexane layer deposited yellowish crystals of **5**, mp 133–135°

General Procedure for 6 and 7. 17α-Acetoxy-6α-methylpregn-4-ene-3,20-dione 3-Phenylhydrazone (6).—17α-Acetoxy-6α-methylprogesterone (1 g) was treated with 250 mg of phenyl-