CHEMISTRY OF ZEARALENONE AND SOME OF ITS DERIVATIVES

Mohammed T. Shipchandler

Research Department
Commercial Solvents Corporation
Terre Haute, Indiana 47808, USA

Abstract

The chemistry of zearalenone (1), a metabolite of Gibberella zeae (Fusarium graminearum) is reviewed. Isolation, structure determination, chemical and physical properties, total syntheses, biosynthesis, and biological properties of zearalenone and some of its derivatives are discussed, and a summary of spectral and analytical methods used is presented.

1. Introduction
2. Nomenclature
3. Structure Determination
4. Naturally Occurring Zearalenone Derivatives
5. Chemical Reactivity, Synthetic Modifications, and Protective Groups
6. Total Syntheses
7. Spectral and Analytical Techniques
8. Fermentation
9. Biosynthesis
10. Metabolites Related to Zearalenone
11. Pharmacology
12. References

1. Introduction

The events leading to the isolation of zearalenone (1) parallel those leading to the discovery of the well-known drug dicoumarol.[1] Several groups of workers, as early as 1928, reported that marked physiological changes in the sex organs of swine were associated with ingestion of corn infected with mold. For example, McNutt et al.[2] investigated a syndrome called vulvovaginitis involving inflammation of the vulva, and the posterior portion of the vagina. A diet involving moldy corn was strongly implicated. Several such studies[3] of inflammed genital organs which followed led Stob et al.[4] to reinvestigate these findings in 1962. This group of researchers was successful in producing vulvar hypertrophy, occasional vaginal eversion, and enlarged mammary glands in female pigs, and swelling of prepuce and mammary glands in male pigs, by feeding moldy corn. A fungus responsible for causing this syndrome was cultured and identified as Gibberella zeae (Fusarium graminearum). A crystalline substance, melting at 161-63°, isolated from such cultures was partially characterized.[4,5] This substance showed uterotropic activity in mice, and improved growth rate and feed efficiency in sheep.[4,5] Since then, others have reported its isolation and characterization.[6,7] Urry, Wehrmeister, Hodge, and Hidy[8] in 1966 carried out a complete characterization, assigned structure 1, and the trivial name "zearalenone". Compound 1 is now produced commercially by fermentation,[9] for use in the manufacture of zeranol

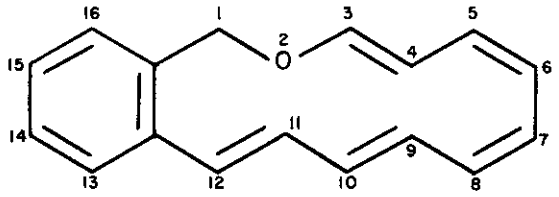
(zearalanol) (68 , Table 6) by catalytic hydrogenation.[10] Zeranol[11] is a commercial anabolic agent used to improve growth rate and feed efficiency in feedlot cattle.[12] Compound 68 as well as its epimer (70, Table 6) are being tested clinically as new drugs for alleviating menopausal and postmenopausal syndromes.[13,14]

This review is the result of a critical survey of the chemical literature on zearalenone and some of its derivatives through early 1975. A 1971 review included some of the biological aspects of this group of compounds.[15]

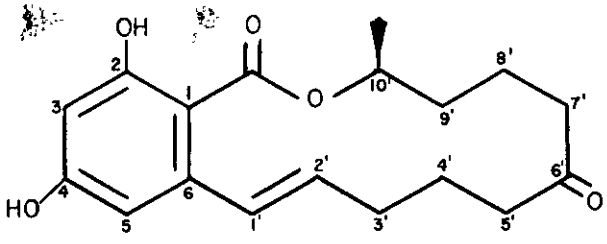
2. Nomenclature

Zearalenone (1) is indexed by Chemical Abstracts as [S-(E)]-3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1H-2-benzoxacyclotetradecin-1,7(8H)-dione; the heterocyclic ring being 1H-2-benzoxacyclotetradecin (129, Chart 1). Early investigators[8] described 1 as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone. A numbering system based upon the β-resorcylic acid system (1, Chart 1) is commonly used.[7,16-23] The size of the macrolide ring is now indicated by the term μ-lactone.[18,21,23,25] An alternate nomenclature based upon the benzoate system (65, Chart 1) is used for naming zearalenone derivatives, including seco-hydroxy acids.[21-25] Terms derived from "zearalenone" to indicate alterations of the functional groups are also used to describe closely related derivatives,[7,8,17,19-22,26] e.g., zearalenol[8,17] for compound 36 or 37 (Table 3) and

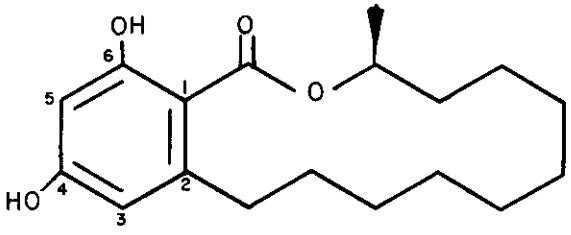
CHART I



129



1



65

dideoxyzearalane[26] for 61 (Table 5). Chemical Abstracts nomenclature has not yet found general acceptance. In this review, in deference to common usage, the numbering (1, Chart 1) based upon the β-resorcylic acid system and the names derived from "zearalenone" will be used.

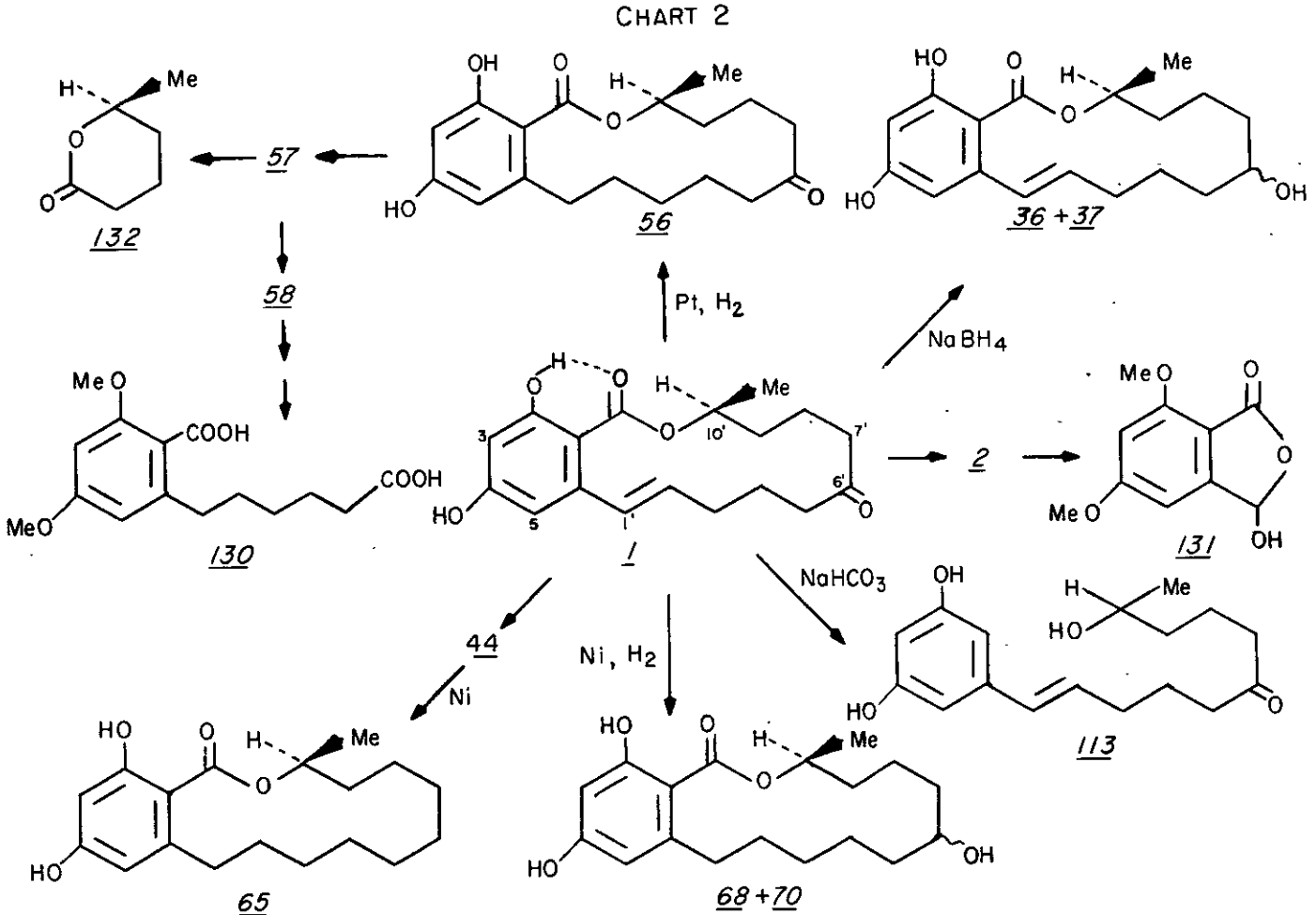
Trivial names used to describe zearalenone include FES[10,27] Fermentation Estrogenic Substance[10] and F-2.[15] It is suggested that these be abandoned in favor of the name "zearalenone" as a common name for 1. The term RAL (Resorcylic Acid Lactone) has been applied to the general group which includes zearalenone and related compounds.[27]

3. Structure Determination

Urry et al.[8] assigned structure 1 to zearalenone using conventional chemical methods (Chart 2) aided by spectral techniques. Several reduction products (36 + 37, 56, 68 + 70, and 65) (Chart 2) involving the olefinic and/or ketonic functionality were prepared using different reducing agents. Zearalenone gave diacetate 13, dimethyl ether 2, two monomethyl ethers 3 and 4, (Table 1) oxime 34, and 2,4-dinitrophenylhydrazone 35 (Table 3).

Degradation of dimethyl ether oxime 58, via a Beckmann rearrangement, gave β-resorcylic acid derivative 130 (Chart 2). Ozonolysis of zearalenone dimethyl ether (2) produced 4,6-dimethoxyphthalaldehydic acid (131). Base hydrolysis (10% $NaHCO_3$) cleaved the macrolide ring, with decarboxylation, producing 113.

CHART 2


Me
H
O
O
57
132
OH
O
H
Me
HO
O
56
HO
O
H
Me
O
HO
OH
36 + 37
58
Me O
COOH
COOH
MeO
130
Pt, H2
NaBH4
H
O
O
H
Me
O
10'
7'
3
6'
HO
5
1'
1
2
Me O
O
O
Me O
OH
131
NaHCO3
OH
H
Me
HO
HO
O
113
44
Ni
Ni, H2
HO
O
H
Me
O
HO
65
HO
O
H
Me
O
HO
OH
68 + 70

An S-configuration was assigned to 1 by two independent methods.[28] Hydroxy ester 125 derived from zearalenone had[24] an S-configuration as determined by Horeau's method.[29] Exhaustive oxidation of 56 produced (S)-5-hydroxyhexanoic acid δ-lactone (132, Chart 2) and (S)-1,4-pentanediol.[22]

4. Naturally-Occurring Zearalenone Derivatives

In 1972, Bolliger and Tamm[7] characterized four minor metabolites of a zearalenone-producing strain of G. zeae. Extensive spectral analyses identified them as 5-formylzearalenone (15), 8'-hydroxyzearalenone (19), 8'-epi-hydroxyzearalenone (21), and 7'-dehydrozearalenone (100). 5-Formylzearalenone (15) and isomeric 3-formyl-zearalenone (17) were synthesized from 1. Oxidation of epimeric hydroxy compounds 19 and 21, as their respective dimethyl ethers 20 and 22, gave 8'-oxozearalenone dimethyl ether (23).

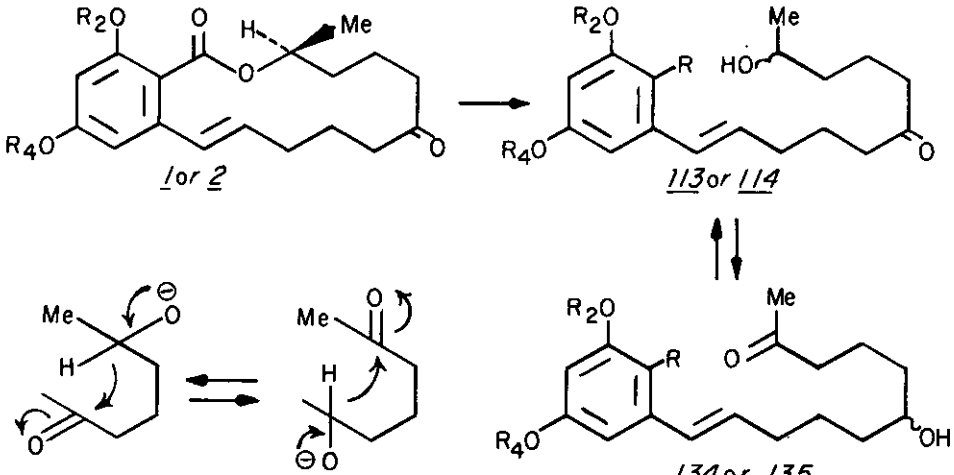
Earlier, Mirocha et al.[15,30] reported isolation of several partially characterized metabolites, collectively called the F-5 series, from G. zeae. Compounds F-5-3 and F-5-4, the two most abundant components, were characterized as epimeric 3'-hydroxyzearalenones on the basis of their spectral and chemical properties.[15] Jackson et al.[31,32] revised this structural assignment to show that F-5-3 and F-5-4 were, in fact, epimeric 8'-hydroxy derivatives of zearalenone (19 and 21) characterized earlier by Bolliger

and Tamm.[7] Comparison of melting points indicates that F-5-3 is identical with 8'-hydroxyzearalenone (19), and F-5-4 with 8'-epi-hydroxyzearalenone (21). A direct comparison of these compounds has not been reported.

5. Chemical Reactivity, Synthetic Modifications, and Protective Groups

A number of analogs of 1, listed in Tables 1-12 along with their physical properties, were synthesized. The lactone function of 1 was quite resistant to acid hydrolysis.[22] Base treatment (10% NaOH), however, resulted in the opening of the macrolide ring with subsequent decarboxylation to give isomeric hydroxy ketones 113 and 134 (Chart 3).[8] An internal hydride shift through a 6-membered transition state was proposed to account for the production of 134. This reaction when carried out in deuterium oxide showed a disappearance,[33] in the proton magnetic resonance (pmr) spectrum, of the aromatic signals of 113 and 134, the methyl doublet of 113, the methyl singlet of 134, and the methylene multiplets alpha to the ketonic functions of 113 and 134. Two sharp singlets of approximately equal area, due to the methine protons attached to the hydroxyl carbons of 113 and 134, were visible. Decarboxylation did not occur when dimethyl ether (S)-2 was hydrolyzed; the resulting product was (R,S)-114. The presence of isomeric compound 135, however, could not be detected. An indirect proof for the existence of an

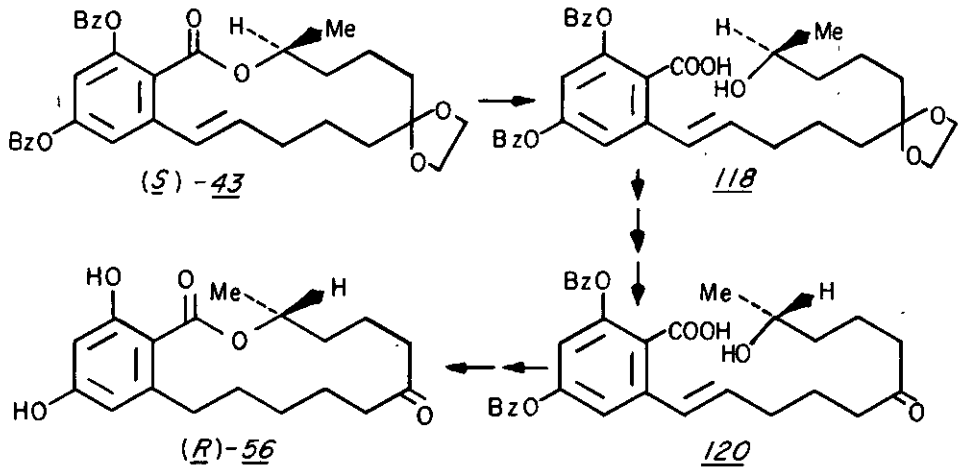
CHART 3



1, 113, 134, R = R_2 = R_4 = H

2, 114, 135, R = COOH, R_2 = R_4 = Me

CHART 4



equilibrium between 114 and 135 was provided. This racemization was prevented when the 6'-oxo function was protected by an acetal formation. Thus, hydroxy acid (S)-116 resulted when dimethyl ether acetal (S)-42 was hydrolyzed.[24] Peters and Hurd[21] synthesized (R)-zearalanone (56) from (S)-1 via a stereoselective route (Chart 4), in which the macrolide ring was opened, the chiral center was inverted, and the ring was reclosed (1 → 6 → 43 → → 56).

The 6'-oxo group of 1 reacted with hydroxylamine and 2,4-dinitrophenylhydrazine to give 34 and 35, respectively.[8] Reaction with 1,2-ethanediol[18] gave acetal 40; reaction with 1,2-ethanedithiol[8] gave thioacetal 44. The acetal protective group was used extensively during synthetic manipulations.[16,18,21,24,25] A Wittig reaction[31] of dimethyl ether 2 gave 55, which was hydrogenated to 76. Sodium borohydride reduction of 1 gave a mixture of two alcohols 36 and 37 (zearalenols), which were separated by fractional crystallization from acetic acid.[34] Hydrogenation over Raney nickel gave a mixture of alcohols 68 and 70 (zearalanols). The ratio of epimers 68 and 70 in the reaction mixture was controlled under different reaction conditions.[8,36] Urry[35] made configurational assignments to the new chiral center by a pmr method involving shift reagents. Stereochemical correlation (36 → 68 and 37 → 70) of the two pairs of alcohols was established by hydrogenation.[34] Young[37] reported a fractional crystallization

method for separation of alcohols 68 and 70, via corresponding 6'-acetates 69 and 71. Alcohol 70 (mp 145-47°) reported by Urry et al.[8] was believed to be a eutectic mixture containing 68 (ca. 30%).[38] Fractional crystallization from aqueous acetic acid gave pure 70 (mp 155-57°).[39] Oxidation[40] of alcohols 68 and 70 gave ketone 56. Acetylation of the phenolic groups during oxidation (72 → 73) led to an improved yield.[41] A stereospecific reduction of the 6'-oxo group of several of the derivatives of 1 was reported by Czaja et al.[42]

Jensen et al.[18] prepared zearalenone derivatives substituted in the 7'-position by a regioselective method. Formylation of 6 provided 52 as a major isomer. Synthetic manipulations (52 → 53 → 106 → 92 + 93 →→94) converted 52 to ketone 94 (an isomer of zearalanone, 56). Similar regioselectivity was observed when zearalanone dimethyl ether (57) was formylated. Analogous results were reported by Jackson.[31] Enolization, however, occurred in the C-5' direction during the preparation of enol ester 104 from diacetate of zearalenone (13). Isomeric enol ester 105 was isolated as a minor product.

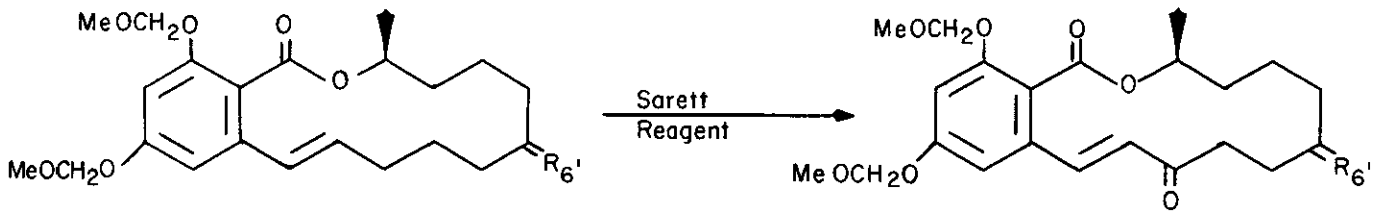
Selective hydrogenation of zearalenone produced zearalanone (56).[8] A number of derivatives in this dihydro-series are listed in Tables 5-8, 10, and 12. Peters[17,43] reported preparation of Z-isomers 96-99 from E-isomers 1, 36, 37, and 54 (Table 9), respectively, by a photochemical method. Inertness of the olefinic function

of 2 towards peracid oxidation was possibly due to the electron withdrawing effect of the lactone carbonyl group.[18] Osmium tetroxide hydroxylation of 6, however, was successful, leading to unstable diol 89. Hydroboration of diether acetal 41 was sluggish.[18] The resulting unstable alcohol yielded ketone 90 on oxidation. Removal of the protective groups of 90 gave dione 91.

Allylic oxidation of 41 and 39 with Sarett reagent gave good yields of ketones 47 and 50, respectively (see Chart 5). Such oxidation of 12 leading to ketone 48 was slow. A mechanistic explanation was provided.[18] Removal of the protective groups of 47 gave dione 49. Sodium borohydride reduction of ketone 47 gave alcohol 51 which on acid treatment gave 101.

Phenolic ethers of 1 and its derivatives were readily prepared.[7,8,18,21,23-26,31] Treatment of 1 with dimethyl sulfate[8] gave a mixture of dimethyl ether 2 and monomethyl ether 4. Diazomethane treatment gave isomeric monomethyl ether 3 only. Similar observations were reported by Jackson.[31] Protective methyl groups were extensively used during synthetic manipulations,[7,8,18,25,31] as well as during total syntheses[16,24,44,45] of 1 and its derivatives. Selective and nonselective removal of the methyl protective groups with boron trihalides was reported.[16,24,44] Benzyl protective groups were used similarly when the olefinic function was absent or when its reduction was desired to be simultaneous with the removal of the protective

CHART 5

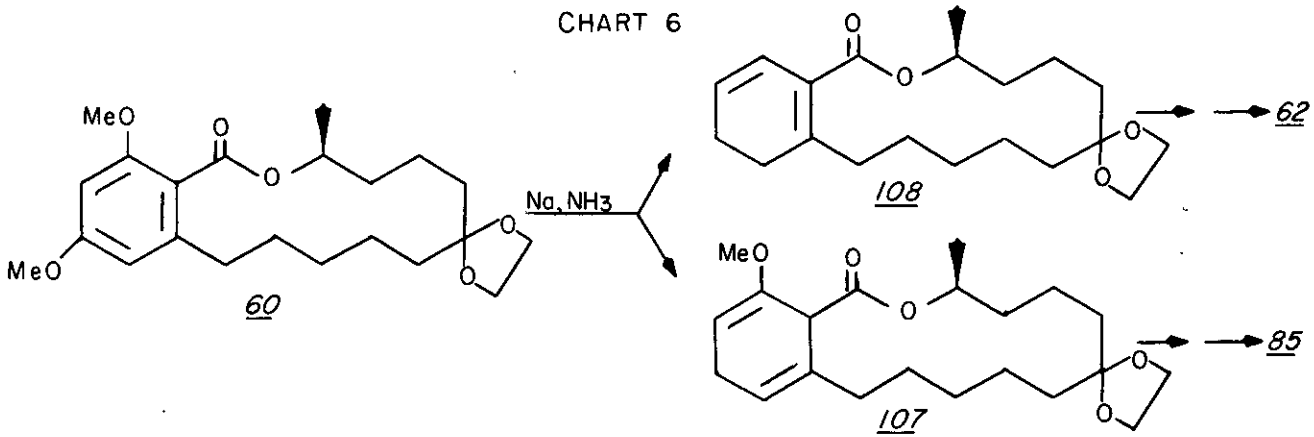


41 ⟶ *47* ($R_{6'}$ = OCH_2CH_2O)

39 ⟶ *50* ($R_{6'}$ = H, OAc)

12 ⟶ *48* ($R_{6'}$ = O)

CHART 6



60

groups.[18,21-23] Selective protection of the 4-hydroxy group of 1 was achieved by tetrahydropyranylation to give monoether 9 in good yield.[23] Benzylation followed by acid treatment, to remove the tetrahydropyranyl group of the uncharacterized product, gave zearalenone 2-O-benzyl ether (7). Phenyltetrazolyl monoethers 8 and 11 and phenyltetrazolyl diether 10 were prepared and hydrogenated to give 4-deoxyzearalanone (88), 2-deoxyzearalanone (87), and dideoxyzearalanone (62), respectively.[23] Earlier, Wehrmeister and Robertson[26] hydrogenolyzed benzoxazolyl diether 67, derived from (S)-zearalane (65), to give (S)-dideoxyzearalane (61). Methoxymethyl protective groups were used by Jensen et al.[15] during chemical modifications of zearalenone (1). Acid treatment was necessary for their removal.

Windholz and Brown[25] synthesized carboxylic acid 26, the single product of a Kolbe-Schmitt reaction, from 1. Chemical degradation unequivocally established the site of carboxylation. A Friedel-Crafts formylation with ethyl formate and aluminum chloride gave 3-formylzearalenone (17). A synthetic sequence gave carboxamide 33 identical with the product derived from carboxylic acid 26. Formylation of 1 with zinc cyanide, hydrogen chloride, and aluminum chloride gave a 2:1 mixture of 3-formylzearalenone (17) and 5-formylzearalenone (15).[7] A Reimer-Tiemann reaction led to a very poor yield of these formylated products.[7]

Nitration[23] of dideoxyzearalanone (62) gave two

isomeric mononitro compounds 77 (major product) and 80. The isomers were separated and reduced to amino compounds 78 and 81, which in turn were diazotized and converted into hydroxy compounds 79 and 82. 5-Aminozearalanone[46] (83) was converted to 5-hydroxyzearalanone (84) via a different route. Quinone 102, characterized as its acetate 103, was an intermediate.[23]

Windholz and Brown[25] studied the Birch reduction of dimethyl acetal 60. One of the two isolated products was dihydro compound 107, (Chart 6), later oxidatively aromatized to 86. Acid hydrolysis removed the acetal protective group of 86, to give ketone 85. Hydrolysis of 107 provided dione 112. The second reduction product, dihydro compound 108, on oxidation followed by hydrolysis gave dideoxyzearalanone (62). It was suggested that during the Birch reduction, methoxyl groups were initially lost giving dideoxyzearalanone acetal 63, which then led to the observed dihydro products 107 and 108. This proposed intermediate (63), synthesized by an independent route, on reduction gave dihydro compound 110 isomeric with the previously isolated compound 108. Base treatment converted 110 to 108. A mechanistic explanation for these observations was provided.[25]

6. Total Syntheses

The unique macrolide structure combined with its interesting biological properties provided stimulus to

several groups of researchers to report total syntheses of 1 and its derivatives. In each instance, construction of the macrolide ring was believed to be the crucial step. The most common approach was synthesis of corresponding hydroxy acids or hydroxy esters followed by lactonization to form the 14-membered ring. Taub et al.[24] and Mullenbach[22] used trifluoroacetic anhydride as the lactonizing agent for hydroxy acids (Charts 7 and 11). Vlattas et al.,[16] (Chart 8) Urry,[50] and Mullenbach[22] used the transesterification method for hydroxy esters. Neither of these techniques, nor a variety of other reagents, gave the desired product 61 from hydroxy acid 122 or hydroxy ester 123. Phosgene and triethylamine finally were used to lactonize 122 (Chart 9).[26] Corey and Nicolaou[49] summarized methods used in the construction of macrocyclic rings, and introduced an efficient, mild, and high-yielding method for lactonization of hydroxy acids including 121. Hurd and Shah[19] introduced the Dieckmann condensation, catalyzed by sodium bis(trimethylsilyl)amide, in the synthesis of macrocyclic ketones, which included (R,S)-zearalanone (56) (Chart 12).[20] Mullenbach[22] used intramolecular "stitching" with thexylborane followed by carbonylation in the synthesis of 56 (Chart 11). In the patent literature, Cross et al.[51] reported a synthesis, which involved cleavage of compound 172, followed by ring closure to zearalenone (Chart 13). The same group of researchers[52] utilized an acyloin condensation for the construction of the macrocyclic ring. The lactone function

CHART 7

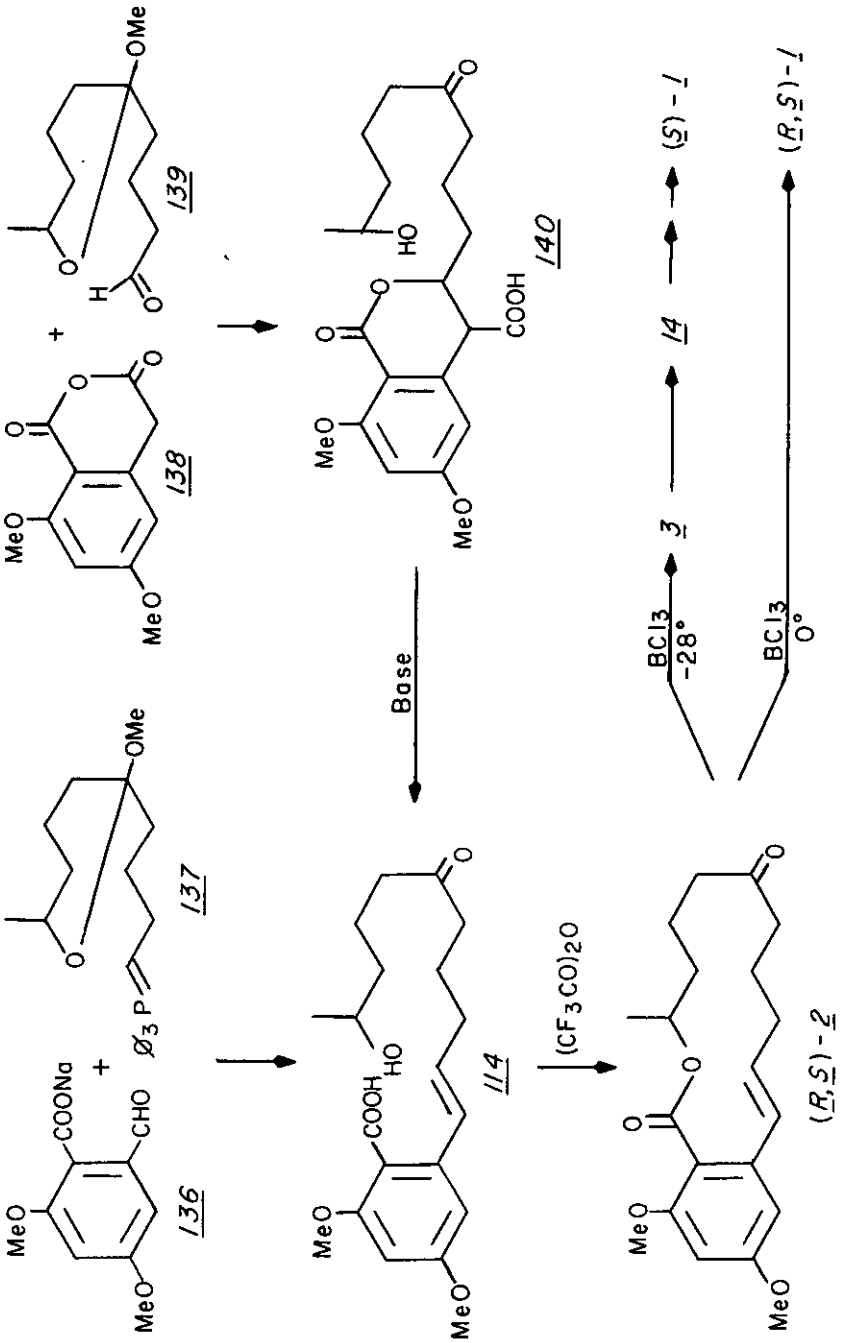
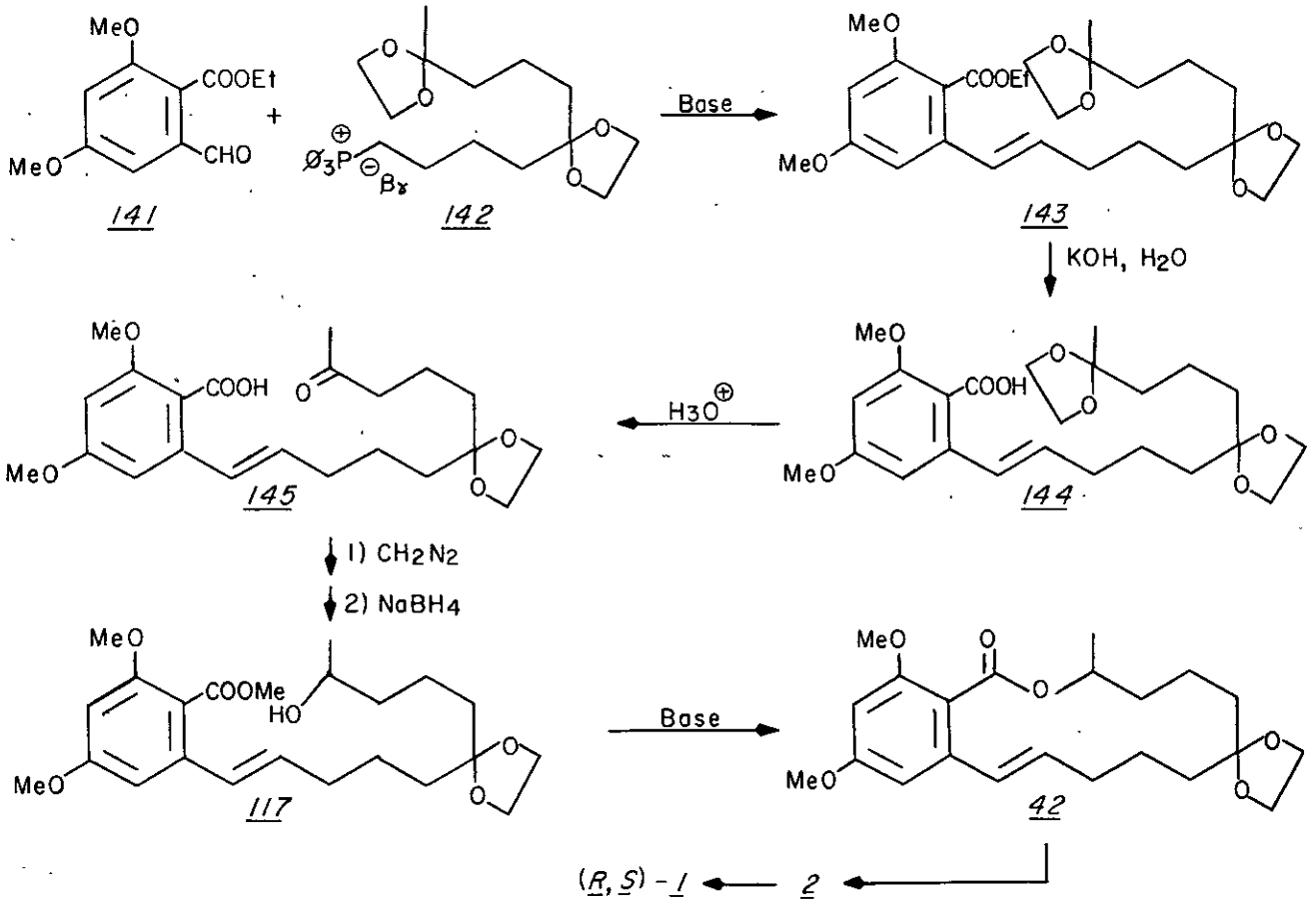

MeO
COONa
CHO
MeO
136
+
Ø3 P
OMe
137
MeO
MeO
138
+
OMe
139
Base
MeO
HO
COOH
140
MeO
COOH
HO
114
(CF3CO)2O
MeO
(R,S)-2
BCl3
-28°
3
14
(S)-1
BCl3
0°
(R,S)-1

CHART 8


MeO
COOEt
CHO
MeO
141
+
$\phi_3P^{\oplus}$ $\ominus$ Br
142
Base
MeO
COOEt
MeO
143
KOH, H_2O
MeO
COOH
MeO
144
$H_3O^{\oplus}$
MeO
COOH
O
MeO
145
1) CH_2N_2
2) $NaBH_4$
MeO
COOMe
HO
MeO
117
Base
MeO
O
O
MeO
42
(R, S) - 1
2

was then generated by the oxidation of benzyl ether 175 (Chart 13).

The method of Taub et al.[24,44] involving bonding of synthons 136 and 137 through a Wittig reaction (Chart 7) to produce hydroxy acid 114. Girotra and Wendler[45] used a Knoevenagel reaction to condense 138 with 139. The resulting product 140 upon base treatment generated hydroxy acid 114. Cyclization gave (R,S)-zearalenone dimethyl ether (2). Demethylation completed total synthesis of (R,S)-1. Selective removal of one of the methyl protective groups gave (R,S)-3 (Chart 7). Resolution followed by deprotection gave (S)-1. The approach of Vlattas et al.[16] was quite similar, which once again involved a Wittig reaction for the construction of the carbon skeleton of zearalenone (Chart 8).

The method of Wehrmeister and Robertson[26] involved condensation of phthalic anhydride (146) with 10-undecenoic anhydride (147) followed by a three-step sequence producing hydroxy acid 122 (Chart 9). Dideoxyzearalane (61), the simplest macrolide having the same heterocyclic ring as zearalenone, resulted upon cyclization.

Urry[50] and Mullenbach[22] generated the β-resorcylate system from aliphatic precursors by modifying a method of Anker and Cook[53] (Charts 10 and 11). Carbon skeletons for the synthesis of 154, 155, 157, 65, and 56 were generated by this method. Condensation of 158 with ethyl acetoacetate (151), for example, gave the cyclic product 159 (Chart 11).

CHART 9

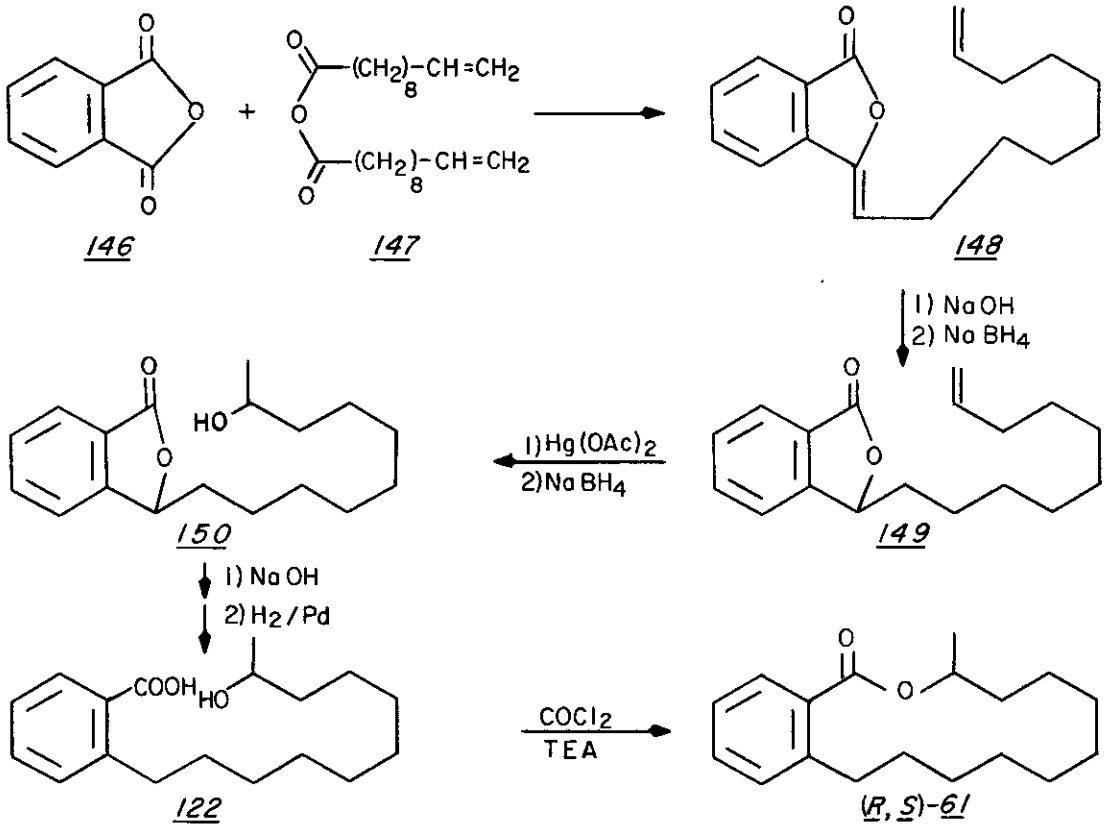

146
147
148
1) NaOH
2) NaBH4
149
1) Hg(OAc)2
2) NaBH4
150
1) NaOH
2) H2 / Pd
122
COCl2
TEA
(R, S)-61

CHART 10

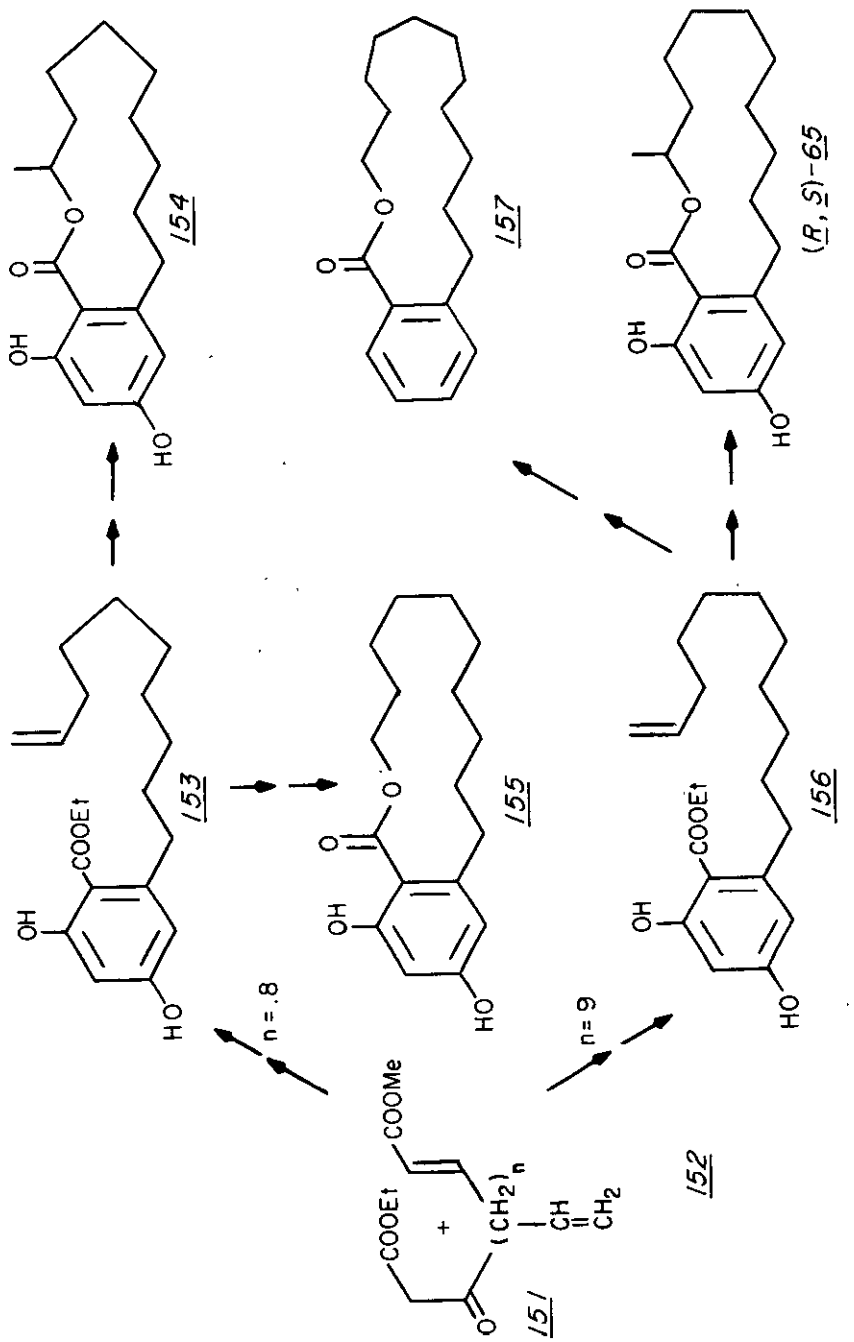
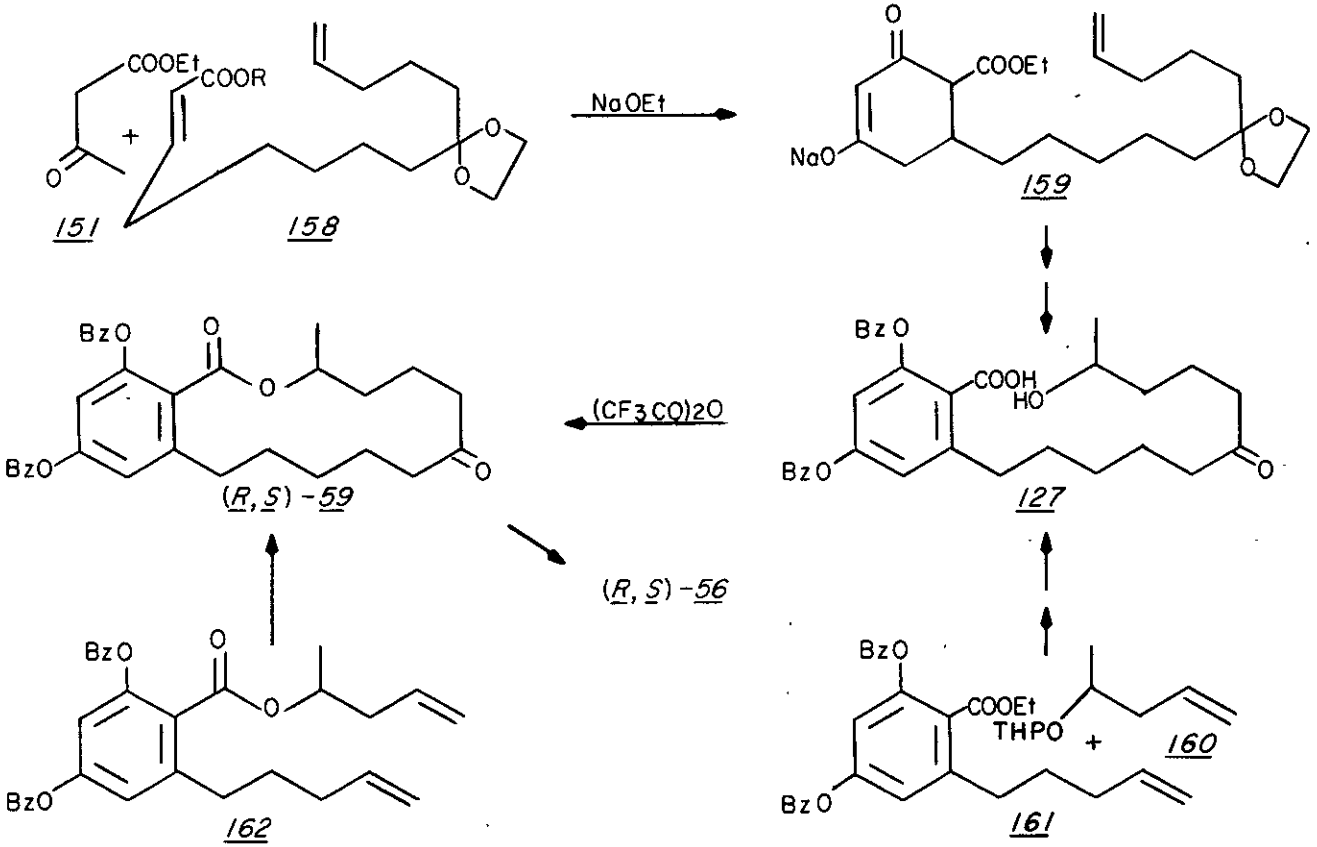


CHART II


COOEt
COOR
151
158
NaOEt
NaO
COOEt
159
BzO
COOH
HO
127
(CF3CO)2O
(R, S) -59
(R, S) -56
BzO
COOEt
THPO
160
161
162
R = CH2CH2OH

Equimolar bromination followed by dehydrobromination generated the desired β-resorcylate system. Hydroboration of the terminal olefinic functions led to primary alcohols which were lactonized to 14-membered macrolide 155 and 15-membered macrolide 157 (Chart 10).[54] Hydration of the olefinic function to secondary alcohols, via the hydroxymercuration procedure, led to the precursors of (R,S)-norzearalane (154, 13-membered macrolide), (R,S)-zearalane (65), and (R,S)-zearalanone (56). Superior yields were realized when the phenolic functions were benzylated during lactonization. An alternate synthesis of 56 was effected by hydroboration[55] of alkenes 160 and 161, followed by carbonylation and oxidation to give ketone 127 (Chart 11). This reaction technique when applied to diene 162 generated the macrocyclic ketone directly.

Hurd and Shah[56] used a Stobbe-type condensation for reacting 163 and 164 (Chart 12). The resulting compound was saponified to acid 165. Esterification with 5-chloro-2-pentanol (166) generated 167, which was hydrogenated to 168. A series of reactions generated diester 169 from 168. Cyclization gave a mixture of β-keto esters 170 (R_5, = COOMe, R_7, = H) and 171 (R_5, = H, R_7, = COOMe). Removal of the carbomethoxy and the benzyl groups completed the total synthesis of (R,S)-zearalanone (56).[19,20]

A synthetic method, not yet applied to zearalenone derivatives, was reported by Bagli and Immer.[57] Medium-sized benzoic acid lactones were generated by peracid cleavage

CHART 12

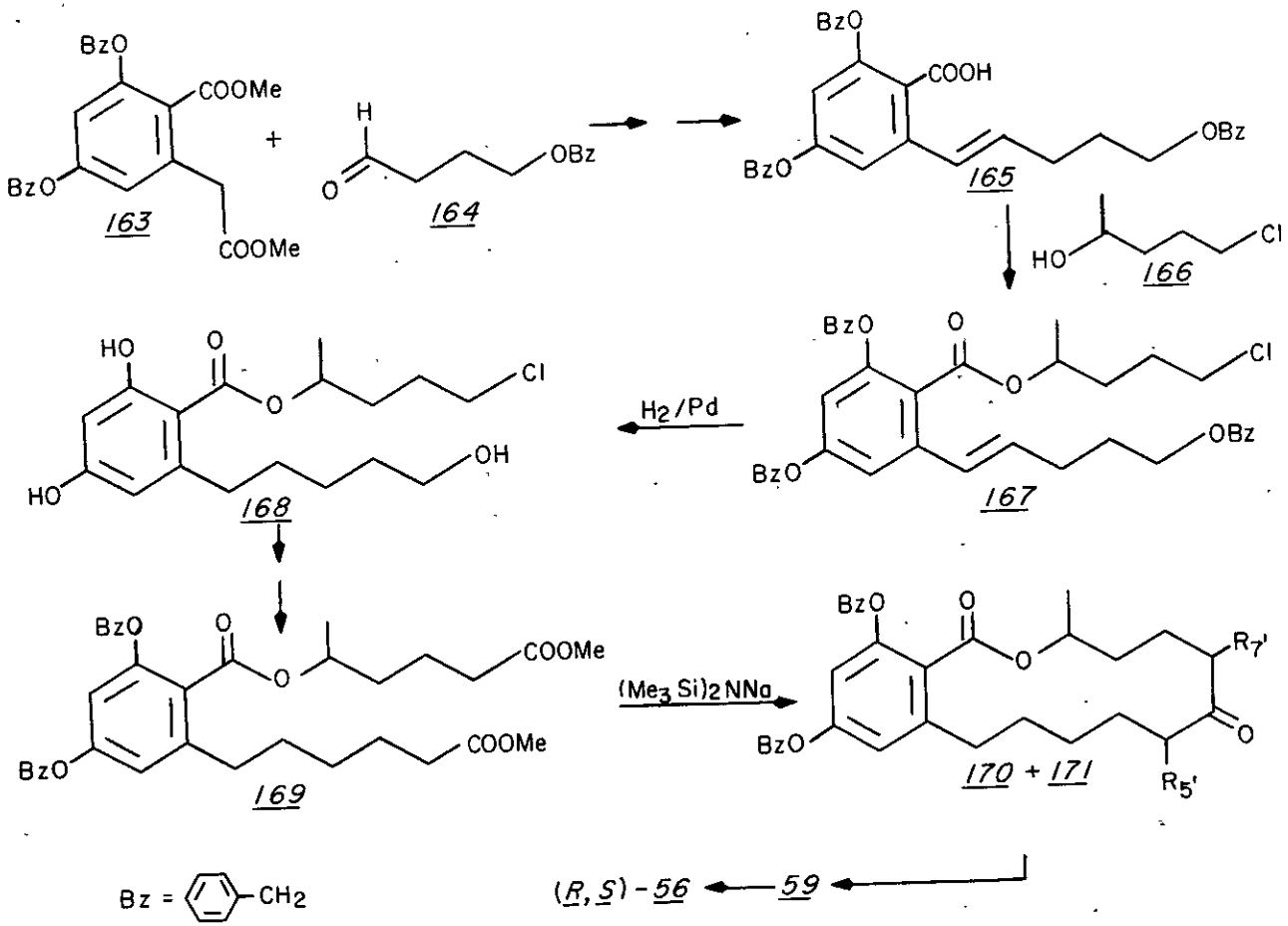
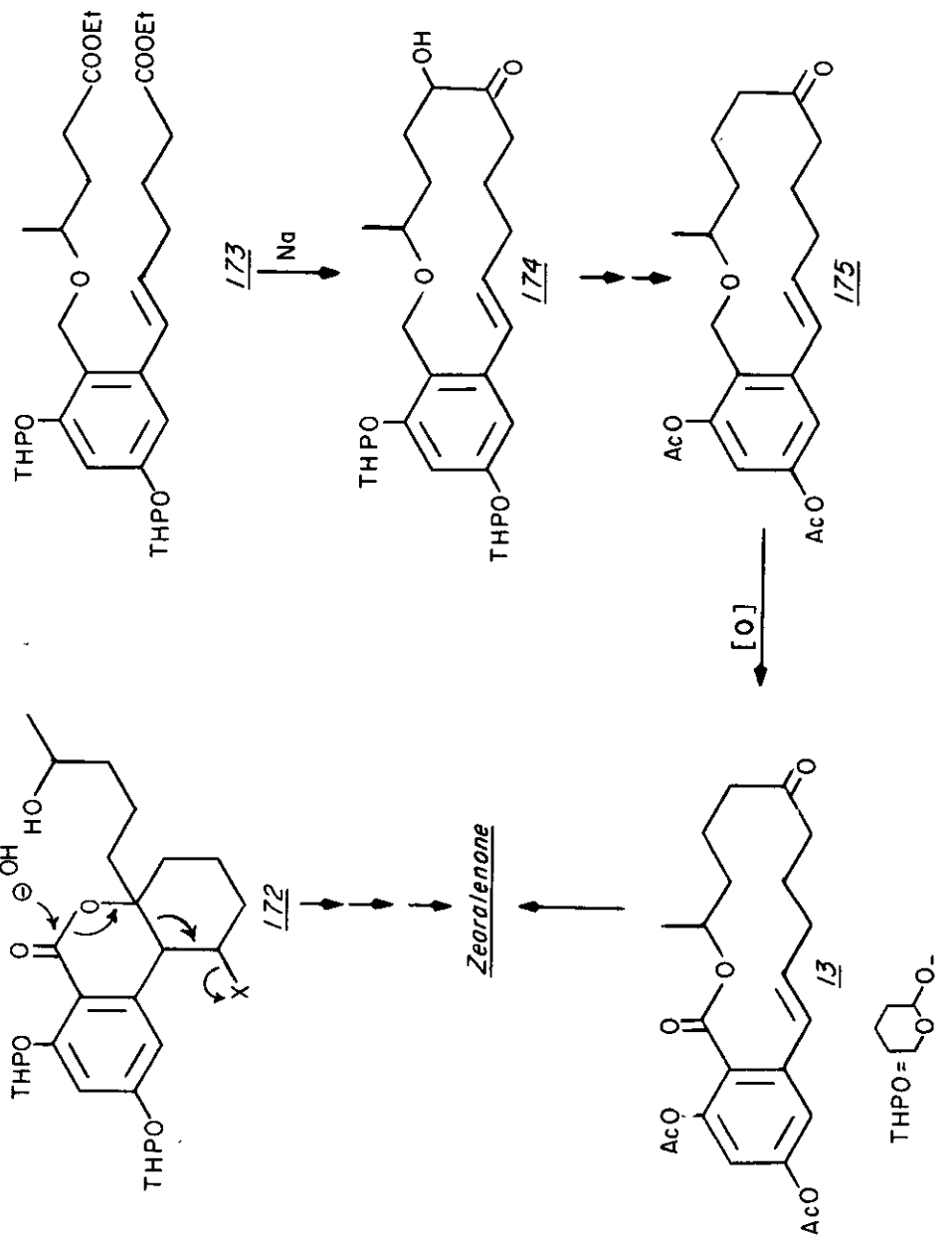

BzO
COOMe
BzO
COOMe
163
+
H
O
OBz
164
BzO
COOH
BzO
OBz
165
HO
Cl
166
BzO
O
O
Cl
BzO
OBz
167
H2/Pd
HO
O
O
Cl
HO
OH
168
BzO
O
O
COOMe
BzO
COOMe
169
(Me3 Si)2 NNa
BzO
O
O
R7'
BzO
O
R5'
170 + 171
Bz = CH2
(R, S) - 56
59

CHART 13


COOEt
COOEt
THPO
THPO
O
173
Na
OH
O
174
THPO
THPO
O
AcO
AcO
175
[O]
HO
OH
O
O
X
THPO
THPO
172
Zearalenone
O
O
O
AcO
AcO
13
THPO=
O
O

of corresponding fused bicyclic systems.

7. Spectral and Analytical Techniques

Ultraviolet (uv), pmr, and mass spectral methods were used by Urry et al.[8] in their investigation of zearalenone structure. Infrared (ir) and uv spectra of 1 were reported by Andrews and Stob[5] and by Mirocha et al.[6b] These spectral techniques were used by many of the investigators in structure determination of zearalenone derivatives.[7,18,25,31,32]

In the pmr spectrum[7,8] of 1 (solvent $CDCl_3$), the hydrogen-bonded 2-hydroxy proton and the 4-hydroxy proton appear as sharp singlets at 12.1 and at 7.3 δ, respectively. The down field resonance (11.1-12.1 δ, solvent $CDCl_3$ or CD_3COCD_3) is observed[7] for 2-hydroxy protons of 19, 21, and 100. This resonance is helpful in distinguishing 4-O-substituted isomers from 2-O-substituted ones.[31] The absence of hydrogen-bonding absorption in the ir spectrum, and the shift of the lactone carbonyl band from 1645 (in the zearalenone spectrum) to 1710 cm^{-1}, is also helpful. 4-O-Substituted derivatives of 1, in general, appear as fluorescent spots on thin layer chromatograms (tlc) (Eastman Silica gel No. 6060) under uv light.[31] 2-O-Substituted and 2,4-di-O-substituted derivatives appear as nonfluorescent spots. Derivatives of salicylic and β-resorcylic acids behave in a similar fashion. A hydroxy group ortho to the carbonyl function is necessary for the fluorescence. Caution

is recommended as other factors, undoubtedly, influence this property.[31]

Another important feature of the pmr spectrum of 1 is the resonance of the 1'-olefinic proton with a splitting constant (J) value of 16 Hz indicating an E-configuration (Z-isomer 96, J = 11.5 Hz).[8,17] A band at 968 cm^{-1} in the ir spectrum supports this assignment.[5,6b,31] Bolliger and Tamm[7] used the J value in assigning an E-configuration to the 7'-olefinic function in 7'-dehydrozearalenone (100). As mentioned earlier, extensive pmr technique was used in structural assignments for isomeric formylzearalenones (15 and 17), metabolites 19, 21, and 100, and their derivatives.

The mass spectrum[8] of 1 shows a prominent molecular ion peak m/e 318, with major peaks at 189, 188 (base), 161, 151, 125, and 112. Bolliger and Tamm[7] studied fragmentation pathways in high-resolution mass spectra of 1 and several of its derivatives. The carbonyl groups of the macrolide ring serve as the sites for α- and β-cleavages. Similar studies were reported by Jackson et al.[31,32] who supported the fragmentation pathways by attaching a bromo group tag on the aromatic ring.

The circular dichroism spectrum[58] of 1 (c = 0.011, MeOH, 24°) gave these values: θ_{350} 0°; θ_{320} -0.020 (shoulder); θ_{290} -0.068; θ_{287} 0; θ_{274} +0.080; θ_{263} 0; θ_{257} -0.047; θ_{252} 0; θ_{246} +0.078; θ_{230} -0.041.

Gas-liquid chromatographic methods for this group

of compounds as their trimethylsilyl ethers were reported.[6b,31,32;59] Several groups of workers reported tlc detection methods for zearalenone.[6b,60-62] Fishbein and Falk[63] summarized these chromatographic methods. Recently, Seitz et al.[64] indicated that the tlc methods, when used for the detection of 1 in spoiled grain, can lead to erroneous conclusions. The behavior of alternariol monomethyl ether, a metabolite of Alternaria is very similar.

8. Fermentation

For early studies, 1 was produced in stationary cultures of G. zeae (F. graminearum) grown on moist sterile corn.[4] A chemically defined medium supported on vermiculite was subsequently developed to improve the recovery and to scale up the production.[65] An economically feasible large scale industrial fermentation process was made possible by isolation of a mutant, (obtained following exposure to a chemical agent) which produced 1 in high titers by a submerged fermentation process.[9,66]

Surveys of Fusarium species for strains capable of synthesizing 1 were made by several investigators.[62,67,68] A possible physiological role of 1 as a sex-regulating hormone in F. roseum was investigated by Wolf et al.[69]

9. Biosynthesis

A polyketide pathway, involving nine acetates, appears to be responsible for biogenesis of zearalenone and

metabolites 15, 19, 21, and 100. Experiments using compounds labeled with radioisotopes indicated ready incorporation of acetate, and dimethyl malonate into zearalenone by cultures of F. roseum. Kinetic studies and degradation of labeled zearalenone supported the polyketide pathway.[70]

Biosynthesis of curvularin, a related macrolide derived from eight acetate units, was studied by Birch et al.[71] A general pathway for 1 and related macrolides was proposed by Bagli and Immer.[57a]

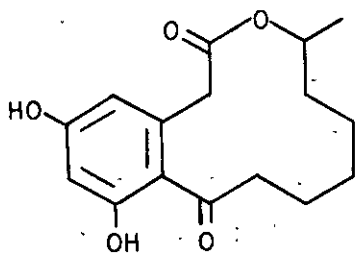
10. Metabolites Related to Zearalenone

Mold metabolites (Chart 14) related to zearalenone are curvularin (176) produced by Curvularia species,[71] lasiodiplodin (177) and de-O-methyllasiodiplodin (178) produced by Lasiodiplodia theobromae,[72] radicicol (monorden) (179) produced[73] by Nectria radicicola and Monosporium bonorden, diaporthin (180) produced by Endothia parasitica,[74] and cladosporin (181) produced by Cladosporium cladosporioides.[75] A total synthesis of (R,S)-di-O-methyl-curvularin was reported by Baker et al.[76]

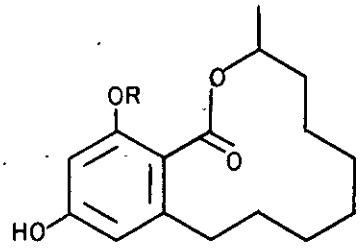
11. Pharmacology

Zearalenone and several of its derivatives exhibit very weak estrogenic activity. For example, zearalenone (1) and zearalanone (56) were 0.1% as active as diethylstilbestrol (DES) in an oral uterotropic assay in rats.[23] Removal of the phenolic functions, as in 62, 87, and 88, reduced the

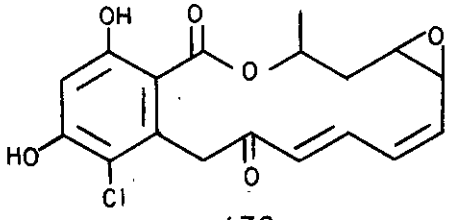
CHART 14



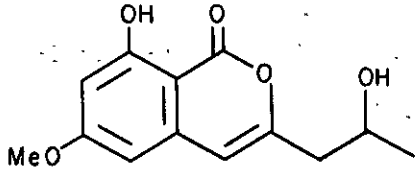
176



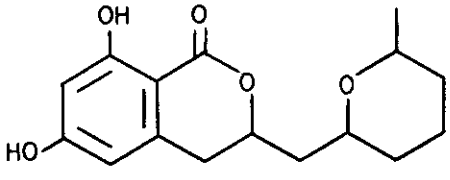
177, R = Me
178, = H



179



180



181

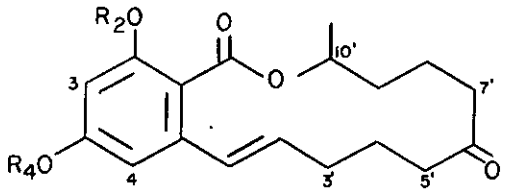
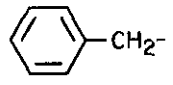


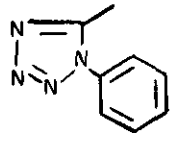
Table 1

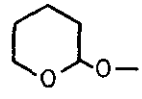
Compd	R_2	R_4	Mp, °C	[α], λ, solvent, c, temp°C	Configuration at C-10'	Ref
1	H	H	164-65	-170.5°, 546, MeOH, 1, 25	S	8
			163-64	-173.8°, 546, MeOH, 1, 24		7
				-134.2°, D, MeOH, 1, 24		
				-191·8°, D, $CHCl_3$, 1, 24		
			188-90	-	R,S	24
2	Me	Me	112-14	-	S	8
			113-14	+49.8°, D, $CHCl_3$, 1, 24		7
				+24.7°, D, MeOH, 1, 24		
			107-10	+25.0°, D, MeOH		24
			124-26	-	R,S	24
			128-29	-		16
3	H	Me	120-22	-	S	8
			120-22	-176.7°, D, $CHCl_3$, 1, 24		7
			108-11	-	R,S	24

Table 1 (contd)

Compd	R_2	R_4	Mp, °C	[α], λ, solvent, c, temp°C	Config-uration at C-10'	Ref
4	Me	H	190-92	-	S	8
5	Et	H	-	-	S	31
6	Bz	Bz	130-32	-	S	18
			128.0-29.5	-		21
			128.0-29.5	-	R	21
7	Bz	H	165-67	-	S	23
8	Bz	PT	159-61	-	S	23
9	H	THP	159-60	-	S	23
10	PT	PT	166-68	-	S	23
11	PT	THP	160-65	-	S	23
12	$MeOCH_2$	$MeOCH_2$	140.5-43.0	-	S	18
13	Ac	Ac	123-25	-	S	8
14	MOA	Me	123-25	-38.5°, D, $CHCl_3$	S	24

Bz = 

PT = 

THP = 

MOA = (-)-Menthoxyacetyl

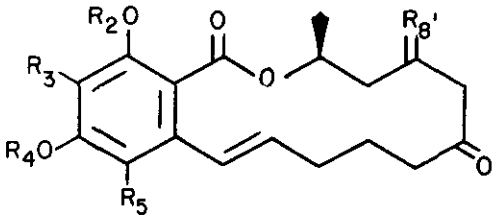


Table 2

Compd	R_2=R_4	R_3	R_5	$R_{8'}$	Mp, °C	$[\alpha]_D^{24}$, (c = 1), solvent	Ref
15	H	H	CHO	H_2	188-90	-42.0°, $CHCl_3$	7
16	Me	H	CHO	H_2	111-12	+52.7°, $CHCl_3$	7
17	H	CHO	H	H_2	171-72	-130.2°, $CHCl_3$	7
					160-64	-	25
18	Me	CHO	H	H_2	113-15	+75.2°, $CHCl_3$	7
19	H	H	H	H,OH	210-12	-149.1°, Me_2CO	7
					198-99	-	32
20	Me	H	H	H,OH	162-64	+11.3°, $CHCl_3$	7
21	H	H	H	H,OH	172-74	-53.1°, Me_2CO	7
					168-69	-	32
22	Me	H	H	H,OH	128-30	+80.4°, $CHCl_3$	7
23	Me	H	H	O	135-44	-93.2°, $CHCl_3$	7
24	H	H	H	H,Cl	-	-	32
25	H	H	H	H,Cl	-	-	32

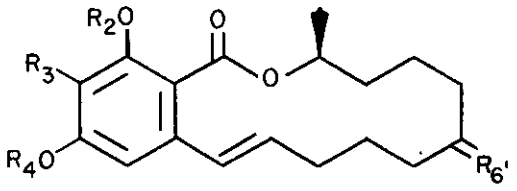
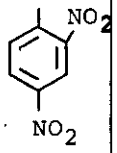
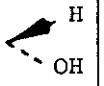
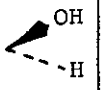


Table 3

Compd	$R_2 = R_4$	R_3	R_6'	Mp, °C	Ref
26	H	COOH	O	158-60	25
27	Me	COOMe	O	111-13	25
28	Ac	COOH	O	crude	25
29	Ac	COOMe	O	crude	25
30	H	CNOH	O	180-82	25
31	Ac	CN	O	73-75	25
32	H	CN	O	120-22	25
33	H	$CONH_2$	O	180-82	25
34	H	H	NOH	210-11	8
35	H	H	NNH 	283-85	8
36	H	H		168-69	34
37	H	H		174-76	34
38	$MeOCH_2$	H	H,OH	partially crystalline	18
39	$MeOCH_2$	H	H,OAc	semisolid	18

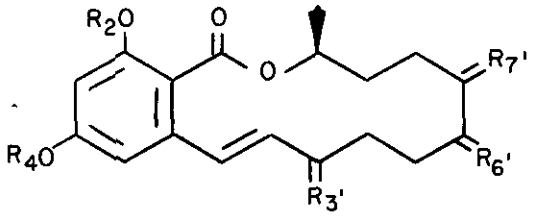


Table 4

Compd	$R_2 = R_4$	R_3'	R_6'	R_7'	Mp, °C	Ref
40	H	H_2	OCH_2CH_2O	H_2	glass	18
41	$MeOCH_2$	H_2	OCH_2CH_2O	H_2	70-72	18
42	Me	H_2	OCH_2CH_2O	H_2	101-03	24
43	Bz	H_2	OCH_2CH_2O	H_2	glass	21
44	H	H_2	SCH_2CH_2S	H_2	-	8
45	Me	H_2	H,OH	H_2	oil	31
46	Me	H_2	H,Cl	H_2	-	31
47	$MeOCH_2$	O	OCH_2CH_2O	H_2	glass	18
48	$MeOCH_2$	O	O	H_2	117.0-19.5	18
49	H	O	O	H_2	214.5-16.0	18
50	$MeOCH_2$	O	H,OAc	H_2	-	18
51	$MeOCH_2$	H,OH	OCH_2CH_2O	H_2	glass	18
52	Bz	H_2	O	CH-OH	gum	18
53	Bz	H_2	O	CH-O 	glass	18
54	H	H_2	H_2	H_2	164.0-65.5	47
55	Me	H_2	CH_2	H_2	73-75	31

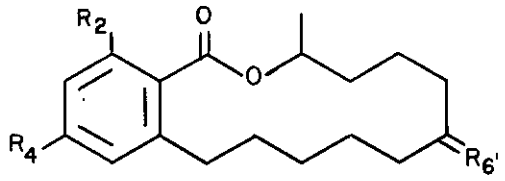


Table 5

Compd	R_2=R_4	R_6'	Mp, °C	$[\alpha]^{25}$,λ, MeOH	Config-uration at C-10'	Ref
56	HO	O	192-93	-	S	8
			190-91	-34.0°,D		21
			190-91	+36.8°,D	R	21
			207.0-08.5	-	R,S	20
			208-10	-		22
57	MeO	O	124.0-25.5	-	S	8
58	MeO	NOH	130-32	-	S	8
59	BzO	O	104	-	S	19
			104	-	R,S	19
			103-05	-		22
60	MeO	OCH_2CH_2O	91-93	-	S	25
61	H	H_2	oil	+92.0°,546, (c = 1)	S	26
			oil	-	R,S	26
62	H	O	89-91	-	S	23
63	H	OCH_2CH_2O	61-63	-	S	25
64	MeO	OCH_2CH_2O	91-93	-	S	25
65	HO	H_2	154-56	-	S	8
			145-47	-	R,S	22
66	BzO	H_2	179-80	-	R,S	22

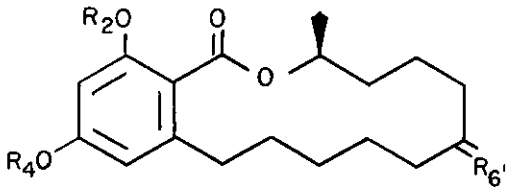
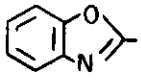


Table 6

Compd	$R_2 = R_4$	R_6'	Mp, °C	$[\alpha]_D^{25}$ (c = 1, MeOH)	Ref
67	BZOX	H_2	120.5-22.5	-	26
68	H	H, OH	182-83	-	8
			178-80	+46°	39
69	H	H, OAc	133-34	-	37
70	H	OH, H	145-47	-	8
			155-57	+39°	39
71	H	OAc, H	163-64	-	37
72	Ac	OH, H	119-20		41
73	Ac	O	153-54	-	41
74	Me	H,OH	-	-	31
75	Me	CH_2	78-81	-	31
76	Me	Me,H	oil	-	31

BZOX = 

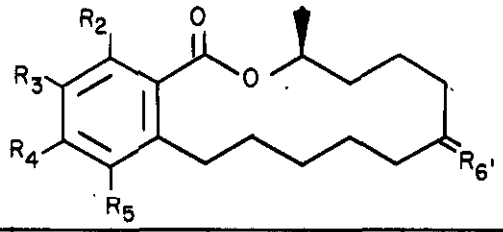


Table 7

Compd	R_2	R_3	R_4	R_5	$R_{6'}$	Mp, °C	Ref
77	H	NO_2	H	H	O	108-10	23
78	H	NH_2	H	H	O	139-40	23
79	H	HO	H	H	O	130-33	23
80	H	H	H	NO_2	O	-	23
81	H	H	H	NH_2	O	-	23
82	H	H	H	HO	O	124-127	23
83	HO	H	HO	NH_2	O	190-93	46
84	HO	H	HO	HO	O	210-15	23
85	MeO	H	H	H	O	96-97	25
86	MeO	H	H	H	OCH_2CH_2O	-	25
87	H	H	HO	H	O	168-70	23
88	HO	H	H	H	O	101-04	23

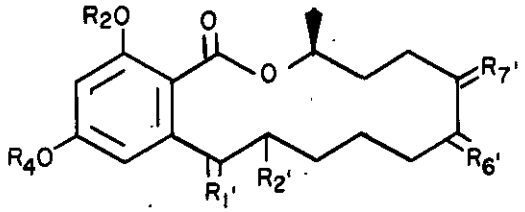
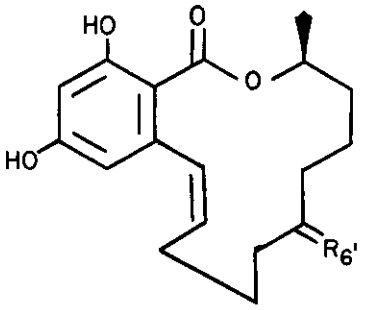


Table 8

Compd	$R_2 = R_4$	R_1'	R_2'	R_6'	R_7'	Mp, °C	Ref
89	Bz	H,OH	OH	O	H_2	-	18
90	$MeOCH_2$	O	H	OCH_2CH_2O	H_2	glass	18
91	H	O	H	O	H_2	172.5-74.5	18
92	H	H_2	H	H_2	CHO,H	148-52	18
93	H	H_2	H	H_2	CHO,H	141-46	18
94	H	H_2	H	H_2	O	168.0-70.5	18
95	H	H_2	H	H_2	COOH,H	-	48

Table 9

Compd, mp in °C, (ref)



96, R_6' = O, 134-35, (17)

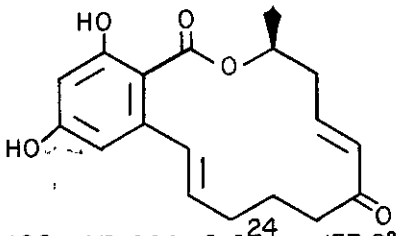
97 =  H, 124-28, (17)

98 =  OH, 126-31, (17)

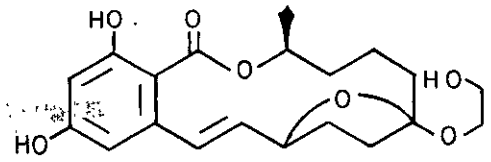
99 = H_2, 143-45, (43)

TABLE 10

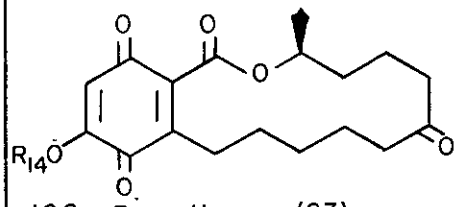
Compd, mp in °C, etc. (ref)



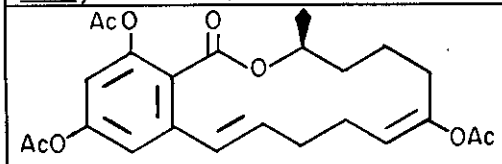
100, 197-200, $[\alpha]_D^{24}$ = -133.8°, (*c* = 1, $CHCl_3$), (7)



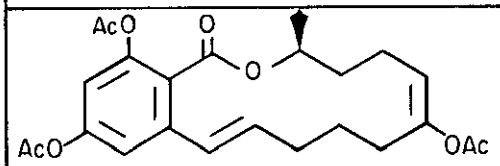
101, 245-46, (18)



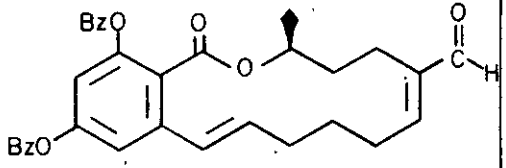
102, R_{14} = H, gum, (23)
103, = Ac, 133-36, (23)



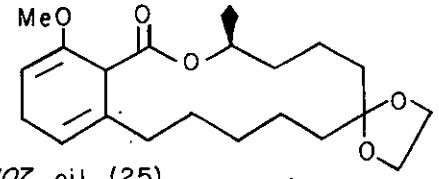
104, 124-25, (18)



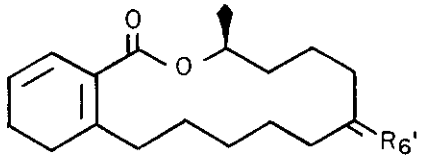
105, 178.5-80.0, (18)



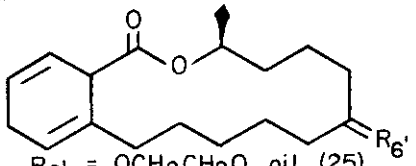
106, glass (18)



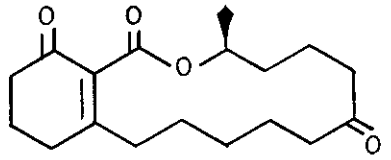
107, oil, (25)



108, $R_{6'}$ = OCH_2CH_2O, oil (25)
109, = O, (25)



110, $R_{6'}$ = OCH_2CH_2O, oil, (25)
111, = O, (25)



112, 48-50, (25)

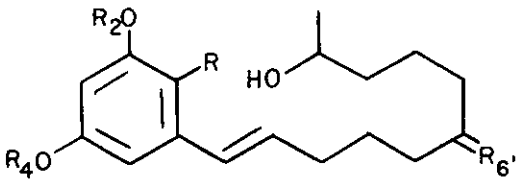


Table 11

Compd	R_2	R_4	$R_{6'}$	R	Mp, °C	Configuration	Ref
113	H	H	O	H	89-91	R,S	8
114	Me	Me	O	COOH	oil	R,S	24, 45
115	Me	Me	O	COOMe	-	R,S	24
116	Me	Me	OCH_2CH_2O	COOH	oil	S	24
117	Me	Me	OCH_2CH_2O	COOMe	oil	S	24
					-	R,S	16
118	Bz	Bz	OCH_2CH_2O	COOH	104-06	S	21
					104-06	R	21
119	Bz	Bz	OCH_2CH_2O	COOMe	oil	S	21
120	Bz	Bz	O	COOH	82-84	R	21
121	H	THP	OCH_2CH_2O	COOH	-	R,S	49

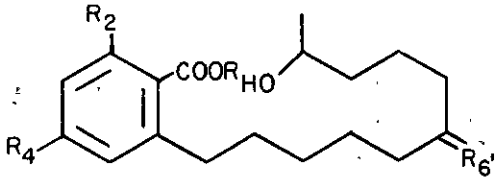


Table 12

Compd	$R_2 = R_4$	$R_{6'}$	R	Mp, °C	Configuration	Ref
122	H	H_2	H	oil	S	26
				oil	R,S	26
123	H	H_2	Me	oil	S	26
				oil	R,S	26
124	MeO	OCH_2CH_2O	H	63-67	S	24
125	MeO	OCH_2CH_2O	Me	oil	S	24
126	BzO	H_2	Et	semisolid	R,S	22
127	BzO	O	H	-	R,S	22
128	BzO	OCH_2CH_2O	Et	-	R,S	22

activity. 5-Hydroxyzearalanone (84) was also less active.[23] Stereochemical correlation of the C-10' chiral center, and the 1'-olefinic function of zearalenone and its derivatives, with mouse uterotropic activity was reported by Hurd et al.[20,21] and Peters.[17] Brooks et al.[48] showed that zearalane (65) was 0.01% as uterotropic as DES in rats. Introduction of a -CHO or a -COOH group in the 7'-position (92, 93, 95, and its 7'-epimer) increased the activity severalfold. Anti-implantation activity of this group of compounds was also reported.[48]

As mentioned earlier, compounds 68 and 70 are in clinical trials.[13,14] The use of zeranol (68) in veterinary practice was discussed by Brown,[77] Burger et al.,[78] and Sharp and Dyer.[79] Metabolic studies on 68 in steers were also reported.[80]

Acknowledgment

The author wishes to thank Mr. M. C. Bachman, Dr. H. L. Wehrmeister, Mr. W. F. Phillips, Mr. J. Crist, and several of his colleagues for their valuable assistance in the preparation of this article.

12. References

(1) M. A. Stahmann, C. F. Huebner, and K. P. Link, J. Biol. Chem., 1941, 138, 513.

(2) S. H. McNutt, P. Purwin, and C. Murray, J. Am. Vet. Med. Ass., 1928, 73, 484.

(3a) E. M. Pullar and W. M. Lerew, Aust. Vet. J., 1937, 13, 28; (b) J. S. Koen and H. C. Smith, Vet. Med., 1945, 40, 131; (c) B. A. McErlean, Vet. Rec., 1952, 64, 539.

(4) M. Stob, R. S. Baldwin, J. Tuite, F. N. Andrews, and K. G. Gillette, Nature, 1962, 196, 1318.

(5) F. N. Andrews and M. Stob, U.S. Patent 3,196,019, 1965.

(6a) C. M. Christensen, G. H. Nelson, and C. J. Mirocha, Appl. Microbiol., 1965, 13, 653; (b) C. J. Mirocha, C. M. Christensen, and G. H. Nelson, ibid., 1967, 15, 497.

(7) G. Bolliger and Ch. Tamm, Helv. Chim. Acta, 1972, 55, 3030.

(8) W. H. Urry, H. L. Wehrmeister, E. B. Hodge, and P. H. Hidy, Tetrahedron Lett., 1966, 3109.

(9) C. L. Keith, U.S. Patent 3,661,712, 1972.

(10) E. B. Hodge, P. H. Hidy, and H. L. Wehrmeister, U.S. Patent 3,239,345, 1966.

(11) "USAN 10 and the USP Dictionary of Drug Names", 1972. Other terms used to describe this compound are P-1496 and MK-188. It is currently marketed under the trademark RALGRO(R).

(12a) Fed. Regist., 1969, 34, 18243; (b) ibid., 1970, 35 13727.

(13) M. Maqueo T. and J. J. Calderon M., the VIIIth World Conference on Fertility and Sterility, Buenos

Aires, Argentina, November 8, 1974.

(14) W. H. Utian, Brit. Med. J., 1973, 579.

(15) C. J. Mirocha, C. M. Christensen, and G. H. Nelson, in "Microbial Toxins", S. Kadis, A. Ciegler and S. J. Ajil, Eds., Academic Press, New York, 1971, pp. 107-138.

(16) I. Vlattas, I. T. Harrison, L. Tokes, J. H. Fried, and A. D. Cross, J. Org. Chem., 1968, 33, 4176.

(17a) C. A. Peters, J. Med. Chem., 1972, 15, 867; (b) C. A. Peters, Commercial Solvents Corp., personal communication indicated that 36 was a precursor of 97 and 37 of 98. Further purification raised the mp of 97 to 126.5-28.0° and that of 98 to 132.5-33.5°.

(18) N. P. Jensen, R. D. Brown, S. M. Schmitt, T. B. Windholz, and A. A. Patchett, J. Org. Chem., 1972, 37, 1639.

(19) R. N. Hurd and D. H. Shah, ibid., 1973, 38, 390.

(20) R. N. Hurd and D. H. Shah, J. Med. Chem., 1973, 16, 543.

(21) C. A. Peters and R. N. Hurd, ibid., 1975, 18, 215.

(22) G. Mullenbach, Ph.D. Thesis, The University of Chicago, 1971.

(23) D. B. R. Johnston, C. A. Sawicki, T. B. Windholz, and A. A. Patchett, J. Med. Chem., 1970, 13, 941.

(24) D. Taub, N. N. Girotra, R. D. Hoffsommer, C. H. Kuo, H. L. Slates, S. Weber, and N. L. Wendler,

Tetrahedron, 1968, 24, 2443.

(25) T. B. Windholz and R. D. Brown, J. Org. Chem., 1972, 37, 1647.

(26) H. L. Wehrmeister and D. E. Robertson, ibid., 1968, 33, 4173.

(27) "The Merck Index", 8th Edition, Merck and Co., Inc., Rahway, N.J., 1968, p. 1126.

(28) C. H. Kuo, D. Taub, R. D. Hoffsommer, N. L. Wendler, W. H. Urry, and G. Mullenbach, Chem. Commun., 1967, 761.

(29) A. Horeau and H. B. Kagan, Tetrahedron, 1964, 20, 2431.

(30) C. J. Mirocha, C. M. Christensen, and G. H. Nelson, Cancer Res., 1968, 28, 2319.

(31) R. A. Jackson, Ph.D. Thesis, University of Minnesota, 1973.

(32) R. A. Jackson, S. W. Fenton, C. J. Mirocha, and G. Davis, J. Agr. Food Chem., 1974, 22, 1015.

(33) W. H. Urry and J. C. Duggan, The University of Chicago, unpublished data cited in ref. 22.

(34) E. B. Hodge, Commercial Solvents Corp., unpublished results.

(35) W. H. Urry, The University of Chicago, unpublished data.

(36a) E. B. Hodge, U.S. Patent 3,697,548, 1972; (b) E. B. Hodge, U.S. Patent 3,704,248, 1972.

(37) V. V. Young, U.S. Patent 3,687,982, 1972.

(38) V. V. Young, Commercial Solvents Corp., unpublished data.

(39) V. V. Young, U.S. Patent 3,574,235, 1971.

(40) V. V. Young, U.S. Patent 3,839,364, 1974.

(41) V. V. Young, U.S. Patent 3,818,044, 1974.

(42) R. F. Czaja, V. J. Grenda, and E. M. Chamberlin, U.S. Patent 3,704,249, 1972.

(43) C. A. Peters, Belgian Patent 808,879, 1973.

(44) D. Taub, N. N. Girotra, R. D. Hoffsommer, C. H. Kuo, H. L. Slates, S. Weber, and N. L. Wendler, Chem. Commun., 1967, 225.

(45a) N. N. Girotra and N. L. Wendler, Chem. Ind. (London), 1967, 1493; (b) N. N. Girotra and N. L. Wendler, J. Org. Chem., 1969, 34, 3192.

(46a) E. B. Hodge, Commercial Solvents Corp., cited in ref. 23; (b) E. B. Hodge, unpublished data.

(47) Compound 54 was prepared from 1 by a Clemmensen reduction process, F. Kavka, Commercial Solvents Corp., unpublished data.

(48) J. R. Brooks, S. L. Steelman, and D. J. Patanelli, Proc. Soc. Exp. Biol. Med., 1971, 137, 101.

(49) E. J. Corey and K. C. Nicolaou, J. Am. Chem. Soc., 1974, 96, 5614.

(50a) W. H. Urry, U.S. Patent 3,586,701, 1971; (b) W. H. Urry, U.S. Patent 3,631,179, 1971.

(51) A. D. Cross, J. H. Fried, and I. T. Harrison, U.S. Patent 3,535,343, 1970.

(52) A. D. Cross, J. H. Fried, and I. T. Harrison, U.S. Patent 3,585,216, 1971.

(53) R. M. Anker and A. H. Cook, J. Chem. Soc., 1945, 311.

(54) W. H. Urry and P. Singh, The University of Chicago, unpublished data cited in reference 22.

(55) H. C. Brown and E. Negishi, J. Am. Chem. Soc., 1967, 89, 5477.

(56) R. N. Hurd and D. H. Shah, J. Org. Chem., 1973, 38, 607.

(57a) J. F. Bagli and H. Immer, Can. J. Chem., 1968, 46, 3115; (b) H. Immer and J. F. Bagli, J. Org. Chem., 1968, 33, 2457.

(58) T. N. Riley, University of Mississippi, personal communication, 1975.

(59) W. J. A. Vandenheuvel, Separ. Sci., 1968, 3, 151.

(60) R. M. Eppley, J. Ass. Offic. Anal. Chem., 1968, 51, 74.

(61) L. Stoloff, S. Nesheim, L. Yin, J. V. Rodricks, M. Stack, and A. D. Campbell, ibid., 1971, 54, 91.

(62) R. W. Caldwell, J. Tuite, M. Stob, and R. Baldwin, Appl. Microbiol., 1970, 20, 31.

(63) L. Fishbein and H. L. Falk, Chromatogr. Rev., 1970, 12, 42.

(64) L. M. Seitz, D. B. Sauer, H. E. Mohr, R. Burroughs, and J. V. Paukstelis, J. Agr. Food Chem., 1975, 23, 1.

(65) P. Hidy, U.S. Patent 3,580,811, 1971.

(66a) P. H. Hidy and V. V. Young, U.S. Patent 3,580,929, 1971; (b) J. R. McMullen, U.S. Patent 3,661,713, 1972; (c) E. T. Woodings, U.S. Patent 3,661,714, 1972.

(67) C. J. Mirocha, C. M. Christensen, and G. H. Nelson, Appl. Microbiol., 1969, 17, 482.

(68) K. Ishi, M. Sawano, Y. Ueno, and H. Tsunoda, ibid., 1974, 27, 625.

(69) J. C. Wolf and C. J. Mirocha, Can. J. Microbiol., 1973, 19, 725.

(70a) J. A. Steele, J. R. Lieberman, and C. J. Mirocha, Can. J. Microbiol., 1974, 20, 531; (b) J. R. Lieberman, M. S. Thesis, University of Minnesota, 1971.

(71) A. J. Birch, O. C. Musgrave, R. W. Rickards, and H. Smith, J. Chem. Soc., 1959, 3146.

(72) D. C. Aldridge, S. Galt, D. Giles, and W. B. Turner, J. Chem. Soc. (C), 1971, 1623.

(73a) R. N. Mirrington, E. Ritchie, W. C. Shoppee, S. Sternhell, and W. C. Taylor, Aust. J. Chem., 1966, 19, 1265; (b) R. N. Mirrington, E. Ritchie, C. W. Shoppee, W. C. Taylor, and S. Sternhell, Tetrahedron Lett., 1964, 365; (c) F. McCapra, A. I. Scott, P. Delmotte, J. Delmotte-Plaquee and N. S. Bhacca, ibid., 1964, 869.

(74) E. Hardegger, W. Rieder, A. Walser, and F. Kugler, Helv. Chim. Acta , 1966, 49, 1283.

(75) P. M. Scott, W. Van Walbeek, and W. M. McLean, J. Antibiot., 1971, 24, 747.

(76) P. M. Baker, B. W. Bycroft, and J. C. Roberts, J. Chem. Soc. (C), 1967, 1913.

(77) R. G. Brown, J. Am. Vet. Med. Ass., 1970, 157, 1537.

(78a) M. L. Borger, L. L. Wilson, J. D. Sink, J. H. Ziegler, and S. L. Davis, J. Anim. Sci., 1973, 36, 706; (b) M. L. Borger, J. D. Sink, L. L. Wilson, J. H. Ziegler, and S. L. Davis, ibid., 1973, 36, 712.

(79) G. D. Sharp and I. A. Dyer, J. Anim. Sci., 1971, 33, 865.

(80) G. D. Sharp and I. A. Dyer, ibid., 1972, 34, 176.

Received, 14th April, 1975

Note added after the completion of this manuscript:

A personal communication from Prof. Wm. H. Watson, FASTBIOS Laboratory, Texas Christian University, Fort Worth, Texas 76129, indicated that he was successful in assigning an R-configuration to 8'-hydroxyzearalenone (F-5-3) (19) by X-ray crystallography. The sample was provided by Prof. C. J. Mirocha, University of Minnesota.