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Natural compounds of the strobilurin series and their synthetic analogues as cell respiration inhibitors

V V Zakharychev, L V Kovalenko

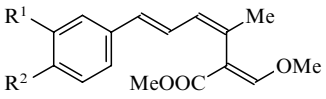
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Abstract. A group of fungicidal antibiotics, β-methoxyacrylic acid derivatives (strobilurins, oudemansins, and myxothiazols), their producers, and mechanisms of action are considered. The fungicidal activity of these compounds is based on the suppression of cell respiration of fungi in the bc_1-complex of cytochromes. They also manifest other biological activities that are not always coupled with inhibition of respiration. Studies of the structure of the natural methoxyacrylates has made it possible to create a novel class of synthetic agricultural fungicides with enhanced stability, high activity, and a broad spectrum of action. The main regularities of the structure – activity relationship and methods of synthesis of these compounds are discussed. The bibliography includes 159 references.

I. Introduction

Some antibiotics produced by various microorganisms (strobilurins, oudemansins, and myxothiazols) were found to inhibit the growth of a broad range of fungi.[1] Mucidin or strobilurin A (**1a**) was the first to be discovered in this group of compounds. Later, several other compounds of similar structure that differed from mucidin only in the number of double bonds and the presence and position of substituents, were identified. These compounds were called strobilurins or oudemansins depending on the structure of their aliphatic chains. All of them are secondary metabolites of various fungi. Myxothiazol **2** has a bacterial origin and its structure differs substantially from that of strobilurin A, although both compounds comprise analogous structural elements.

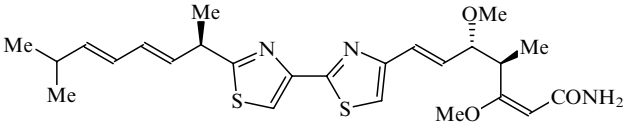


1a – g

$R^1 = R^2 = H$ (**a**); $R^1 = OMe$, $R^2 = H$ (**b**), Cl (**c**);
$R^1 = H$, $R^2 = OMe$ (**d**); $R^1 = OH$, $R^2 = H$ (**e**);
$R^1 = Me_2C{=}CHCH_2O$, $R^2 = H$ (**f**);
$R^1 = OH$, $R^2 = Me_2C{=}CHCH_2O$ (**g**).

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The distinctive feature of this group of compounds is the presence of a fragment of β-methoxyacrylic acid (as methyl ester or amide) linked through an α- or β-carbon atom to the rest of the molecule. They possess identical mechanism of action, which consists in the inhibition of cell respiration at the region of the bc_1 complex of electron-transport cytochromes (complex III). Such an inhibition mechanism made possible wide application of these compounds as a biochemical tool for the study of oxidative phosphorylation. These compounds also served as models for creation of a basically new class of pesticides currently known as 'strobilurin analogues'.

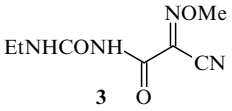
The electron-transport chain of respiratory enzymes plays a crucial role in supplying cells of all aerobic organisms (from bacteria, fungi, and plants to higher animals) with energy. The structure of components of the respiratory chain that are intermediates in the electron transfer from NADH to oxygen, varies insignificantly on passing from species to species. However, substantial differences are observed in the resistance of enzymes of the cytochrome system of various living organisms to specific inhibitors, which is used by certain organisms in the competition struggle.[2, 3]

Natural differences in the structure of respiratory enzymes can be used in the design of agrochemical preparations for achieving their high selectivity with respect to plants and low toxicity for mammals.[2] Owing to the wide occurrence of redox processes involving the cytochrome system, the respiration inhibitors can be used for suppressing the vital activity of a great variety of organisms. Thus many synthetic strobilurin analogues possess not only fungicidal, but also insecticidal, acaridical, and nematocidal activities.

Other natural compounds that incorporate a β-methoxyacrylic acid fragment as a structural element are also known. For instance, a β-methoxyacrylate fragment in dihydrokawain (5,6-dihydro-4-methoxy-6-phenylethyl-2*H*-pyran-2-one), which has been recently isolated from kava *Piper methysticum*[4] and possesses fungicidal activity, includes a hydropyranone ring. However, there is no evidence that this compound inhibits respiration.

The recently discovered derivatives of mesoxalic acid amidonitrile *O*-methyloxime (*e.g.*, cymoxanil **3**) are structurally similar

to some strobilurin analogues. However, their fungicidal activity is associated with blocking of RNA synthesis in fungal cells.[5]

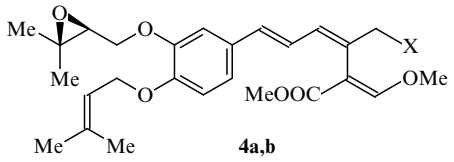


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II. Natural methoxyacrylate-type inhibitors

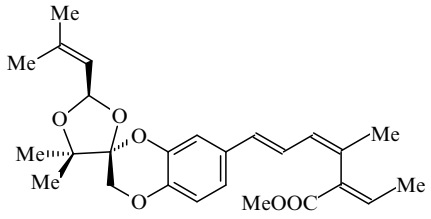
The high fungicidal activity of the new antibiotic mucidin (**1a**) isolated from the cultural medium and mycelium of the fungus *Oudemansiella mucida* was first discovered by Musilek *et al.*[6–8] in the mid-1960's. Two fungicidal antibiotics called strobilurins A and B were isolated from mycelium of the basidiomycete *Strobilurus tenacellus* in 1977.[9] Structural elucidation of these compounds revealed that strobilurin A and mucidin are identical and represent methyl (2*E*,3*Z*,5*E*)-2-methoxymethylene-3-methyl-6-phenylhexa-3,5-dienoate.[10,11] The *E*,*Z*,*E*-configuration of the double bonds of strobilurins was confirmed by chemical and spectroscopic studies[12] as well as by stereospecific synthesis.[13] The spectral properties of a synthetic *E*,*E*,*E*-isomer of strobilurin A[13] differed from those of the natural compound.

Later, several other secondary metabolites of various fungi were isolated and characterised. They are strobilurin A derivatives containing substituents in the benzene ring or in the aliphatic chain (compounds **1b–g**, **4–8**).

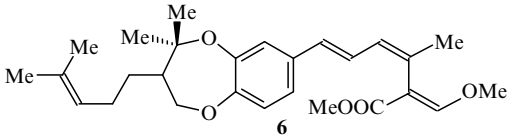


4a,b

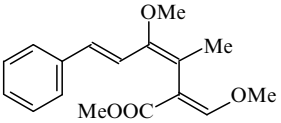
X = H (**a**), OH (**b**).



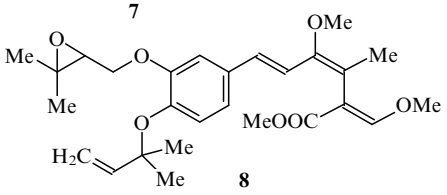
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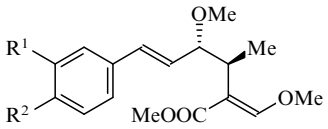
Oudemansins (**9a–c**) possess similar structure. Formally, they can be regarded as the products of addition of methanol at the C(3)–C(4) double bond of strobilurins. The known strobilurins and oudemansins together with their producers are listed in Table 1.

Considering the trivial names of methoxyacrylate-type antibiotics, it should be noted that the letter symbols A, B, and C in the names of strobilurins and oudemansins refer to substituents in the aromatic ring and their positions. The same letters are used for the same substitution pattern [*cf.* the structures of strobilurin B (**1c**) and oudemansin B (**9b**)]. The early name of strobilurin A, mucidin, was preserved in the biochemical literature. Strobilurin X was termed by analogy with oudemansin X,[1] because the investigators who first discovered this compound did not give it any trivial name. Two strobilurins F were identified and named almost simultaneously by independent groups of investigators; therefore, they bear additional numerical indices (in the order of publication).[1]

Table 1. Natural strobilurins and oudemansins.

Structure	Name	Producing organism	Ref.
1a	Strobilurin A (mucidin)	*Oudemansiella mucida*, *Strobilurus tenacellus*, *Bolinea lutea etc.*	6–9, 15–17
1c	Strobilurin B	*S. tenacellus*	9
1f	Strobilurin C	*Xerula longipes*, *X. melanotricha*	16
1d	Strobilurin X	*O. mucida*	18
4b	Hydroxystrobilurin D	*Mycena sanguinolenta*	19
5	Strobilurin E	*Crepidotus fulvotomentosus*	20
4a	Strobilurin D	*Cyphellopsis anomala*	21
1e	Strobilurin F-1	*Cyphellopsis anomala*	21
1g	Strobilurin F-2	*Bolinea lutea*	15, 22
6	Strobilurin G	*B. lutea*	15, 22
1b	Strobilurin H	*B. lutea*	15, 22
7	9-Methoxystrobilurin A [a]	*Favolaschia* spp.	23
8	9-Methoxystrobilurin K [a]	*Favolaschia* spp.	23
9a	Oudemansin A	*O. mucida*	24
9b	Oudemansin B	*Xerula longipes*, *X. melanotricha*	16
9c	Oudemansin X	*O. radicata*	25

[a] Trivial nomenclature is retained.

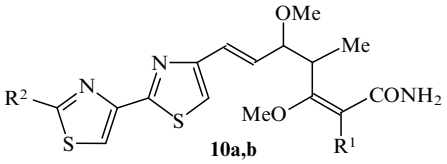


9a–c

$R^1 = R^2 =$ H (**a**); $R^1 =$ OMe, $R^2 =$ Cl (**b**); $R^1 =$ H, $R^2 =$ OMe (**c**).

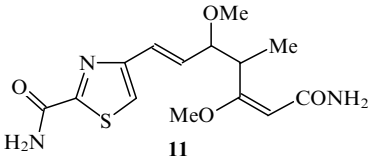
Myxothiazol **2** (or myxothiazol A) mentioned above produced by the myxobacteria *Myxococcus fulvus* is also a derivative of β-methoxyacrylic acid.[14,26,27] In contrast with strobilurins and oudemansins, the β-methoxyacrylate fragment of the myxothiazol molecule is represented by an amide rather than by an ester and is substituted at the β-carbon (but not at the α-carbon) atom. Nevertheless, the mechanism of biological action of this antibiotic is close to that of the natural esters of methoxyacrylic acid.[28,29]

For a long time, myxothiazol had no analogues among β-methoxyacrylate-type compounds. However, a great number of related compounds were discovered recently. At present, 33 myxothiazols have been isolated, and the structures of 24 of them have been given in a review.[1] Unfortunately, comprehensive information about the structure of myxothiazols is still absent in the current literature. It is known only that in myxothiazols B–I, K–O (**10a**) and Q, X, and Y (**10b**), R^2 = MeCO, MeCH(OH), or an (*E*,*E*)-nonadienyl residue containing hydroxy, carbonyl, and epoxide groups. Myxothiazol J does not exist. Myxothiazol P (**11**) has only one thiazole ring. In myxothiazols R–W (**12**), the β-methoxyacrylic fragment is absent and R is an oxygenated three-, five-, or six-carbon-atom chain.[1]

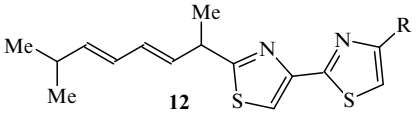


10a,b

R^1 = H (**a**), Me (**b**).



11



12

Strobilurins A and B, all of oudemansins,[1] and myxothiazol A[30] have been synthesised. The generalised data concerning natural methoxyacrylates can be found in a comprehensive review.[1]

The conformer of strobilurin A (**1a**) with a minimum energy was calculated using the molecular mechanics method and the molecular orbital theory. It was found that the phenylpentadienyl and methyl β-methoxyacrylate fragments are planar, but are arranged in perpendicular planes. However, according to these calculations, the energy barrier to rotation around the C(2)–C(3) bond is low, therefore enantiomers cannot be isolated at room temperature.[31]

The structure of oudemansin A **9a** in the crystalline state was determined by X-ray diffraction analysis.[24] It was found that its conformation closely resembles the conformer of strobilurin A with the minimum energy.[31] Apparently these conformations of strobilurin and oudemansin are close to the forms of these molecules bound to cytochrome.[31]

III. Biological activity of the natural compounds

Low concentrations of strobilurins, oudemansins, and myxothiazols inhibit the growth of diverse fungi. Their mechanism of action is associated with violation in electron transport in complex III of the mitochondrial membrane, which results in the inhibition of cell respiration.[28, 29, 32–34]

Complex III (ubiquinone–cytochrome *c* oxidoreductase or the bc_1-complex) is an intermediate link in the chain of respiratory enzymes of bacteria and mitochondria (plant chloroplasts contain a structurally homologous b_6f-complex). It catalyses the electron transfer from hydroquinone to cytochrome *c*. The energy of the redox reaction is thereby converted into a chemiosmotic membrane potential through the translocation of $2e^-$ and $4H^+$ across the membrane.

According to the Mitchell Q-cycle concept,[3, 35, 36] complex III has two quinone reaction centres localised on opposite sides of the membrane. One of them, the so-called Q_o-centre (also designated as Q_p or Q_z), is the site of hydroquinone oxidation by the reaction:

$$2QH_2 \longrightarrow 2Q + 4e^- + 4H^+.$$

Two electrons are transferred by a high-potential route via the FeS protein and cytochrome c_1 to reduce cytochrome *c*. The other two electrons move to the opposite side of the membrane by a low-potential route formed by cytochromes b_H and b_L, to the second quinone centre, the so-called Q_i-centre (also designated as Q_n, Q_c, and Q_r) where the quinone is reduced:

$$Q + 2H^+ + 2e^- \longrightarrow QH_2.$$

The inhibitors binding to the Q_i-centre induce changes in the absorption spectrum of cytochrome b_H (b_{562}). Antimycin is the most specific of these inhibitors. Methoxyacrylates were among the first to be discovered in a group of compounds that block respiration by binding to the Q_o-centre. This binding results in a shift in the absorption band of cytochrome b_L (b_{566}).[29, 32, 33, 37–42]

Since all tissue respiration inhibitors of this type contain a residue of *b*-methoxyacrylic acid (MOA) as a structural fragment, which seems to be the key element for manifestation of biological activity, they were termed MOA-inhibitors.[33]

The binding site of MOA-inhibitors is not identical with the quinone site, since in the presence of these compounds ubiquinone remains bound with cytochrome *b*. This may be accompanied by changes in the relative position of the ubiquinone molecule at the reaction centre due to a conformational rearrangement of the cytochrome and the resulting violation of the electron transport to the FeS protein.[43–45] Thus the concentration of myxothiazol required for 50% inhibition of bovine heart cytochrome *b* activity is 0.58 mol per mol of cytochrome.[29] At high concentrations, MOA-inhibitors can displace the quinone from the site of its binding with the Rieske FeS-protein.[41] Apparently, the binding of inhibitors is reversible, since they can replace one another in the Q_o-centre.[39]

Like methoxyacrylates, other compounds can also bind with the Q_o-centre. Pyricidine (which inhibits also complex I), 6-hydroxy-5-n-undecyl-4,7-dioxobenzothiazole,[2] dibromothymoquinone,[2, 41] and the chromone antibiotics stigmatellins A and B[2, 41, 44, 46] also possess inhibitory activity. Despite the reciprocal competition of these compounds, their binding sites in the Q_o-centre are other than those of MOA-inhibitors.[1, 2]

The structure of the bc_1-complex and the Q_o-centre as well as the mechanisms of resistance to MOA-inhibitors are discussed in a number of publications.[3, 35, 36, 47]

Strobilurins and oudemansins manifest nearly identical high activity *in vitro* against a wide array of fungi,[9, 15, 16, 18, 19–21, 23–25, 48, 49] but are inactive against bacteria. Strobilurin F-1 (**1e**), which manifests much lower activity, is an exception.[21] For example, the minimum inhibitory concentrations of strobilurin A (**1a**) for *Candida albicans, C. crusei, C. parapoilosic, C. tropicalis, Cryptococcus neofarmans, Trichophyton mentagrophytes, Epidermophyton floccosum,* and *Microsporum canis* lie in the range 0.1–12.8 mg ml^{-1}, whereas the inhibitory concentrations for *Aspergillus* spp. and *Scopulariopsis* spp. range from 100 to 1000 mg ml^{-1} (see Ref. 48). Owing to its antimycotic activity, strobilurin A has been used in clinical and veterinary medicine under the commercial name of Mucidermin Spofa.[1]

Myxothiazol A **2** is one of the most active MOA-inhibitors.[32] It manifests not only fungicidal properties, but inhibits also the growth of some Gram-positive bacteria,[1] reversibly inhibits the late G_1/S phase of the cell cycle of lymphoblastic T-cells,[50] and possesses insecticidal activity.[1] In addition, myxothiazol **2** can bind to the phylloquinone site in photosystem I ($K_d = 9.5 \times 10^{-6}$ mol $litre^{-1}$). However, this inhibitor does not manifest any activity *in vivo* due to the higher affinity of phylloquinone for this site.[51, 52] Myxothiazol **2** inhibits also complex I of mitochondria.[2] Other myxothiazols rank below myxothiazol A in fungicidal activity.[1]

Oudemansin A (**9a**),[24] strobilurins A (**1a**), B (**1c**),[9] E (**5**),[20, 49] D (**4a**),[21] and G (**6**)[15] inhibit the growth of human tumour cells, the latter three compounds manifesting the highest activity. 9-Methoxystrobilurins (**7**, **8**)[23] and strobilurin G (**6**)[15] also display cytostatic activity. Strobilurins D (**4a**)[21] and E (**5**)[20, 49] possess antiviral properties. Strobilurins A (**1a**) and B (**1c**) as well as oudemansin A (**9a**) inhibit chitin synthase.[53]

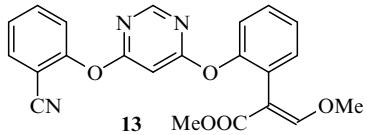
Strobilurins A (**1a**), B (**1c**), C (**1f**), and X (**1d**) as well as oudemansins A (**9a**) and B (**9b**) are relatively non-toxic for mice. According to different data, the peroral lethal dose, LD_{50}, for strobilurin A is 500 mg kg^{-1} (see Ref. 8) or 825 mg kg^{-1} (see Ref. 18). Intraperitoneal LD_{50} is 250 mg kg^{-1} (see Ref. 8); that for oudemansin A exceeds 300 mg kg^{-1} (see Ref. 31). However, myxothiazol **2** is highly toxic for all animals tested (peroral LD_{50} for mice is 2 mg kg^{-1}).[27]

Despite the variety of useful properties, natural MOA-inhibitors have found only limited use as medicinal drugs or biochemical tools for the study of cell respiration. They cannot be used as agricultural pesticides because they have some serious disadvantages. For example, strobilurin A, which possesses high activity against a wide variety of fungi *in vitro*, is fairly inactive in greenhouse studies. This may be due to the low photochemical stability and a relatively high volatility of this compound. Hence, it rapidly evaporates from the surface of leaves of the treated plants. The time of the photochemical loss of the first 50% of strobilurin A in thin film experiments (τ_{50}) is 1 min.[31] Nevertheless, this group of antibiotics presents substantial interest. The relative simplicity of their structure, their ability to retain high activity irrespective of significant changes in their structure, and a basically new mechanism of their action, which entails the absence of cross-resistance in pathogens insensitive to the currently used fungicides, stimulate the synthesis of biologically active analogues of the natural substances. Although respiration inhibitors may be hazardous for homoiothermal organisms, the low toxicity of individual strobilurins and oudemansins suggests that synthetic analogues may exist which differ substantially in toxicity with respect to fungi and homoiothermal organisms.[1]

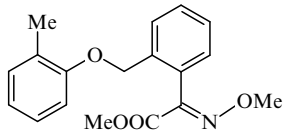
IV. Synthetic strobilurin analogues and structure – activity relationships

Although the first attempts to synthesise analogues of natural MOA-inhibitors were undertaken only in the early 1980's, over 200 patents for the synthesis of these compounds, their practical applications, and composite mixtures, have been published.[31, 54, 55]

Synthetic analogues of strobilurins have been patented as broad-spectrum agricultural and industrial fungicides, nematocides, insecticides and acaricides, plant growth regulators, antitumour and antiviral preparations. The undeniable leaders in this area are the British company ICI and the German company BASF who have succeeded in creating experimental fungicides ICI-5504 (**13**) and BAS-490F (**14**).



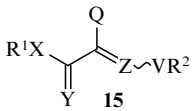
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Two fragments can be distinguished in the molecules of natural MOA-inhibitors and their synthetic analogues, *viz*., a toxophore or a pharmacophore, and a carrier group[56] (or a ballast group[54] or backbone[47]). Thus in strobilurins, the β-methoxyacrylate fragment plays the role of a toxophore and a substituted (*E*,*E*)-phenylpentadienyl residue is the backbone. It is the toxophore that is responsible for the binding with the methoxyacrylate site of the enzyme, although it is inactive if it is not bound with the backbone. In turn, the backbone not only imparts lipophilicity to the compounds, which is needed for the transport of a pharmacophore into the quinone centre, but also enables matching of the shape of the inhibitor molecule and that of the Q_o-centre cavity.[47]

Both factors must be taken into consideration in design of strobilurin analogues. The structure of strobilurins can be modified in two independent ways: (1) substitution of the (*E*,*E*)-phenylpentadienyl chain of strobilurins for various aryl, heterocyclic, and other groups; (2) substitution of the toxophore element, a β-methoxyacrylic acid fragment, for isosteric fragments of crotonic, methoxyiminoacetic, and analogous acids. Synthetic analogues of the natural MOA-inhibitors are compounds with the general formula **15**.



15

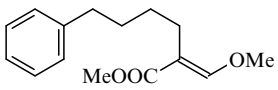
X = O, S, NR^3; Y = O, S; Z = CH, N; V = CR^4, O, S, NR^5; $R^1 - R^5$ = H, Alk; Q = Ar, Het, ArX–, HetX–, *etc.*

In synthetic analogues, Q usually stands for a bulky hydrophobic substituent, which mimics a labile phenylpentadienyl residue (the backbone). This largely determines the biological activity, photostability, selectivity, and systemic properties of a compound. The rest of the molecule is a toxophore. The elements V, X, Y, and Z can vary rather independently. More or less active are esters, amides, and thioamides of appropriately substituted acrylic[57] and crotonic[58] acids, β-alkoxy-,[59] β-alkylthio,[60] β-aminoacrylic acids,[61] *O*-alkyloximes,[62, 63] *S*-alkylthiooximes,[64] and alkylhydrazones of glyoxalic acids[65] and unsaturated acids of analogous structure. Toxophore groups with different structures are also known. For example, compounds **15** devoid of ester or amide groups (R^1 = H, Me, X = CH_2, Y = O, Z = N, V = O, R^2 = Me) are known to inhibit respiration.[47] However, these compounds rank below esters and amides of the above-mentioned acids in activity.

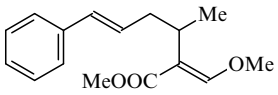
After the discovery of a large variety of compounds that are not derivatives of β-methoxyacrylic acid but are able to inhibit cell respiration, the term 'synthetic MOA-inhibitors' is no longer a correct definition for this group of biologically active substances. It was therefore suggested that such compounds be called 'synthetic strobilurin analogues'.[66]

The pioneering studies in the field of synthesis of strobilurin analogues were aimed at increasing their photostability. The effects of structural changes on the photostability of strobilurin analogues were considered by some authors more comprehensively.[31, 56] Greenhouse studies of oudemansin activity revealed that the presence of a β-methoxyacrylate group did not decrease the photochemical stability of these compounds.[31, 56]

The simplest strobilurin A analogue **16** with partially hydrogenated double bonds and the oudemansin A analogue **17** did not manifest any useful activity in hothouse studies, although compound **17** shows fungicidal properties on agar cultures of fungi.[31]



16



17

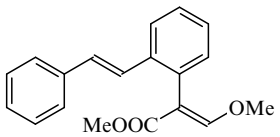
One of the first synthetic analogues of natural MOA-inhibitors was the so-called MOA-stilbene **18**.[57, 67–70] This compound possesses fungicidal and insecticidal properties and surpasses its natural prototypes in activity.[1] X-Ray analysis of a monocrystal of compound **18** showed that the planar methyl β-methoxyacrylate and stilbene fragments are nearly perpendicular to each other as is the case with the strobilurin molecule.[31] Stilbene **18**, like strobilurin A, is a potent inhibitor of mitochondrial respiration (I_{50} for stilbene is 0.04 μM, that for strobilurin A is 0.11 μM[31]). This compound is photochemically more stable than its natural analogue (τ_{50} for irradiation in thin film is 3 min) and less volatile.[56] The photostability of MOA-stilbene increases considerably in the presence of photostabilisers.[56]

In the series of b-alkoxy-a-arylacrylates **19**, even methyl (*E*)-β-methoxy-α-phenylacrylate **19a** (without substituents in the benzene ring) manifests a pronounced fungicidal activity (Table 2).

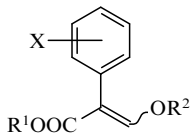
Table 2. Comparative activities of acrylates **19**, **20**, and **21**.[57]

Compound	R^1	R^2	X	Subject of study [a]					
				P.r.[b]	*E.g.*[c]	*V.i.*[d]	*P.o.*[e]	*C.a.*[f]	*P.v.*[g]
E-**19a**	Me	Me	H	4	4	4	0	2	4
Z-**19b**	Me	Me	H	2	0	0	0	0	0
E-**19c**	Et	Me	H	0	0	0	0	0	4
E-**19d**	Me	Et	H	0	0	1	0	0	0
E-**19e**	Et	Et	H	0	0	0	0	0	1
E-**19f**	Me	Me	2-(*E*-PhCH=CH)	4	4	4	3[h]	4	4
E-**19g**	Me	Me	3-(*E*-PhCH=CH)	0	0	0	0	0	3
E-**19h**	Me	Me	4-(*E*-PhCH=CH)	0	0	0	0	0	2
Z-**19j**	Me	Me	2-(*E*-PhCH=CH)	4	4	4	3[h]	4	4
E-**19k**	Me	Me	2-Cl	0	4	0	0	4	4
20	–	–	H	4	3	4	4	4	4
21	–	–	–	3	0	2	2	–	4

Note. The infected plants were sprayed with emulsions (the concentration of active substance was 100 ppm).
[a] Designations: 4 — undamaged plants; 3 — up to 5% damage; 2 — 6% – 25% damage; 1 — 26% – 59% damage; 0 — 60% – 100% damage in comparison with nontreated plants. [b] *P.r.* — *Puccinia recondita* (on wheat); [c] *E.g.* — *Erysiphe graminis* f. sp. *hordei* (on barley); [d] *V.i.* — *Venturia inaequalis* (on apple-tree); [e] *P.o.* — *Pyricularia oryzae* (on rice); [f] *C.a.* — *Cercospora arachidicola* (on peanut); [g] *P.v.* — *Plasmopara viticola* (on grapes); [h] spraying with an emulsion with the concentration of the active substance of 25 ppm.

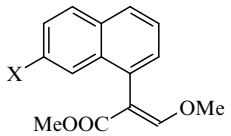


18

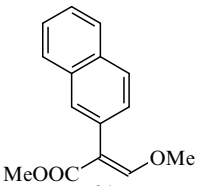


19a – j

On the whole, phenylacrylates **19** are more active for $R^1 = R^2 = Me$ than in those cases where R^1 or R^2 are represented by other alkyl substituents or hydrogen atoms. *E*-Isomers are usually more active than *Z*-isomers; it is probable that the latter, being very weak inhibitors of mitochondrial respiration, acquire the fungicidal activity following isomerisation.[31] Substituents in the nucleus can both strongly enhance the activity and decrease it. Compounds having a bulky substituent similar to the styryl fragment in the strobilurin molecule at position 2 of the phenyl ring are usually more active. The activity of naphthylacrylates strongly depends on the position of a toxophore: α-naphthylacrylate **20** (X = H) is much more active than the β-isomer **21** (see Table 2).



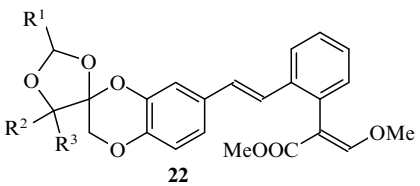
20



21

X = H, Ph, Hal, Alk *etc*.

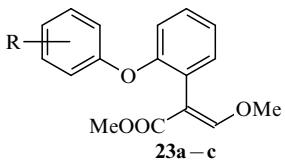
Although substituted MOA-stilbenes manifest high fungicidal activity in hothouses, in field tests they are moderately active due to their low photostability.[1] However, owing to their availability MOA-stilbenes, together with natural compounds, have found wide use as tools in biochemical studies. Of particular interest are stilbenes **22,** which are analogues of strobilurin E and possess antiviral and antitumour properties.[71]



22

In order to increase the photostability of such compounds, it was suggested to hydrogenate the double bond between two benzene rings[72] and to replace it by other spacer groups, such as CH_2O, SCH_2, O, S,[57,73–75] SO, SO_2,[57] CH_2SO, CH_2SO_2,[76] *etc*.[56,77] Methyl (*E*)-methoxyimino-[2-(2-methylphenoxymethyl)-phenyl]acetate (**14**) (BAS-490F), an experimental fungicide produced by BASF and possessing a protective, curative, and eradicating activities is an example. Its standard doses vary from 50 to 350 g ha^{-1} (see Ref. 66).

Acrylate **23a**, a diphenyl ether derivative, is also highly efficient against a great number of fungi.[57,78] This compound is sufficiently resistant to light (τ_{50} for irradiation in thin film is 30 h).[56] In addition, this compound possesses systemic fungicidal activity.[1,54] Compound **23b**, which contains three benzene rings, is even more active,[54,77] but is devoid of systemic properties. Besides, a serious disadvantage of this derivative is that it caused severe damages to some cultures in field tests.[54] The reasons for phytotoxicity of such compounds have not been finally established.[79] A change in the position of a phenoxy group in the molecule results in a sharp decrease in the fungicidal activity. Thus the isomer **23c** is a weak fungicide. In heterocyclic analogues of the ester **23b**, the high level of fungicidal activity is often associated with their ability to migrate in the plant. However, the biological properties of compounds change in an unpredictable manner depending on the number and position of heteroatoms in the rings: sometimes this activity drops drastically, and the action spectrum is narrowed or the phytotoxicity is enhanced.[54] Among derivatives of the heterocyclic series, methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate (**13**) (ICI-A5504), turned out to be the most efficient. This compound is an experimental fungicide and exerts systemic and translaminar activities against asco-, basidio-, deutero-, and oomycetes.[80,81]



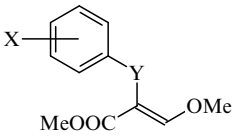
23a – c

R = H (**a**), 3-PhO (**b**), 4-PhO (**c**).

Table 3. Activities of acrylates **24a**–**i**.[82]

Compound	X	Y	Object of study [a]					
			P.r.	*E.g.*	*V.i.*	*P.o.*	*C.a.*	*P.v.*
24a	2-Ph	CH_2	1	0	3	0	0	0
24b	3-Ph	CH_2	4	3	4	0	3	4
24c	4-Ph	CH_2	0	0	0	2	0	0
24d	H	NMe	0	4	4	4	1	4
24e	3-Ph	NMe	4	4	1	3	4	4
24f	H	O	0	0	2	0	0	0
24g	3-Ph	O	4	0	4	3	3	4
24h	H	S	0	0	0	3	0	3
24i	3-Ph	S	4	4	4	2	4	4

Note. The infected plants were sprayed with emulsions (the concentration of the active substance of 100 ppm).
[a] For designations see footnote to Table 2.

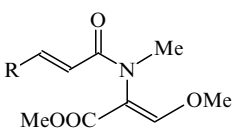


24a–**i**

In a series of compounds of the formula **24** the toxophoric group of which is attached to an aryl or a heterocyclic residue not directly, but through O and S atoms or through NR or CR_2 groups, the derivatives with *meta*-substituents in the aromatic ring display the highest activity (Table 3).

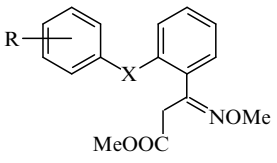
A vast array of structurally diverse synthetic analogues of strobilurin are presently known. Thus biological activities of a great number of compounds with the general formula **15** (where Q designates various substituted carbo- and hetero-cycles), such as derivatives of indole,[83] pyrazole,[84–86] pyrrole [87–89] (methoxyacrylate derivatives of pyrrole have been described in the review [55]), triazine,[90] dibenzo[*b*,*e*]-1,4-dioxin,[91] thiazole,[92–94] isoxazole,[92] pyridine,[95–98] pyrimidine,[99] *etc*., have been assayed. The structure–activity relationships for this group of compounds are nearly the same as those for α-phenylacrylates. Thus the activity of monocyclic compounds increases as certain substituents are introduced at the position adjacent to the toxophoric group. As in the case of α-phenylacrylates, the β-styryl fragment and the radicals having similar bulk and shape, enhance the activity.

Methyl methoxyacrylates derived from amides of aromatic and heteroaromatic acids **25** possess fungicidal properties.[100, 101] In some cases, esters **26** manifested higher activity than the corresponding derivatives of glyoxylic acid.[102] Fungicidal activity was also found in compounds **27** with an unusual structure of the toxophoric group.[103, 104]

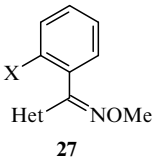


25

R = Ar, Het.

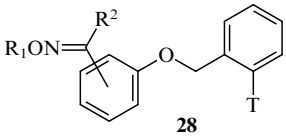


26



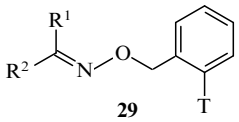
27

Het is oxazol-2-yl, 1,3,4-oxadiazol-2-yl, *etc*.



28

T is the toxophore



29

Certain oximes **28**[105, 106] and **29** [107–119] show enhanced fungicidal activity in comparison with their analogues that do not contain an oxime fragment in their backbone. The spatial isomerism about the C=N bond may be crucial for the manifestation of activity of these compounds.[120]

In the series of synthetic analogues of the natural MOA-inhibitors, the experimental fungicides ICI-A5504 (**13**) and BAS-490F (**14**) have been studied in most detail. Like the natural compounds, these analogues bind with the Q_o-centre of cytochrome *b* and actively inhibit cell respiration.[66, 80, 81, 121, 122] Both substances have low toxicity in rats (LD_{50} > 5000 mg kg^{-1}).

The reason for such a selectivity are the peculiarities of their biokinetic properties rather than their selective affinity for the molecular target. Thus the inhibition constants of complex III from various living organisms were determined using various methoxyacrylates and their analogues.[121] The pI_{50} values for MOA-stilbene (**18**), BAS-490F (**14**), and myxothiazol (**2**) are given in Table 4. The oxidase from cereals was the least sensitive to all of the inhibitors tested. The difference in the pI_{50} values is less pronounced for enzymes from other species. Structural changes in compounds that enhance their activity against fungi, induced analogous changes in the activities against other species.

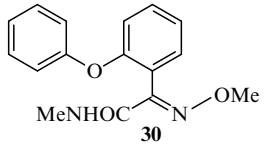
Table 4. pI_{50} Values for inhibitors with respect to complex III of various organisms.[121]

Compound	Yeast	Botrytis	Fly	Rat	Corn
MOA-stilbene (**18**)	7.3	7.4	7.8	7.6	6.3
BAS-490F (**17**)	7.9	7.8	6.9	6.3	6.0
Myxothiazole (**2**)	8.0	7.9	8.3	7.8	6.5

Analysis of biokinetic properties of a series of compounds revealed that such properties as absorption, transport, and metabolism are more important for their selective toxicity. For example, the high activity of compound **14** against surface-located powdery mildew is due to its diffusion through the gaseous phase and the low level of its absorption by leaves in which it is rapidly metabolised. In contrast, an analogue of this preparation in which the ester group is replaced by the methylamide group is expected to be highly active against endoparasitic fungi.[123]

A recent report deals with *N*-methyl-(*E*)-methoxyimino-(2-phenoxyphenyl)acetamide (code SSF-126) [124] (**30**), a new preparation of this series, which possesses a broad spectrum of fungicidal activities and is being tested. It is assumed that the

main area of its application is the fight against paddy rice diseases.[125]



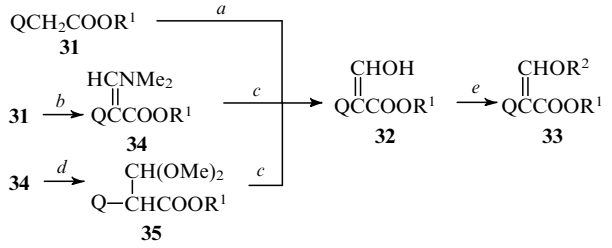
30

Some strobilurin analogues can also bind with the b_6f-complex of chloroplasts in the Q_o or Q_i centre. Thus compounds **18** and **23a**,**b** bind with the Q_i centre, whereas compounds **24b**,**e**,**g**, with the Q_o centre. No binding was observed for compounds **20** (X = Ph) and **25** (Ar = 2- or 4-ClC_6H_4).[126]

V. Synthesis of strobilurin analogues

Syntheses of β-alkoxyacrylates are usually based on esters of substituted acetic acid **31**. Condensation with formates in the presence of a base gives aldehydoesters **32**.[31, 54, 56, 81] The reaction with dimethylformamide dimethylacetal gives compounds **34**, which also yield aldehydoesters **32** following hydrolysis or alcoholysis. Alkylation of enolates (compounds **32**) results in β-alkoxyacrylates **33** (Scheme 1).

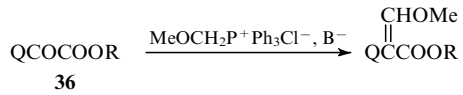
Scheme 1



(*a*) HCOOR, NaH; (*b*) $Me_2NCH(OMe)_2$, Py·TsOH; (*c*) H_2O, H^+;
(*d*) MeOH, H^+; (*e*) B, R^2X (X = Hal, SO_3OR^2);
R^1, R^2 = Alk; Q = Ar, Het, ArX–, HetX–, *etc*.

β-Methoxyacrylates are often synthesised from glyoxalates **36** by the Wittig reaction [31, 56] (Scheme 2).

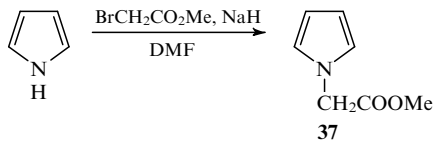
Scheme 2



36

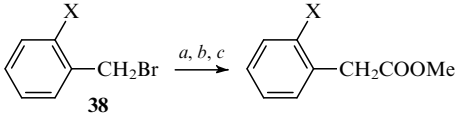
Synthesis of the corresponding derivatives of acetic (**31**) or glyoxylic (**36**) acids is carried out by conventional methods.

Alkylation of nitrogen-containing heterocycles with methyl bromoacetate in the presence of bases was used, *e.g.*, in the synthesis of pyrrole derivatives **37**.[55, 58]



37

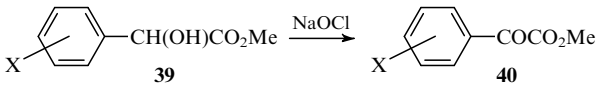
Reaction of benzyl halides and their *ortho*-substituted derivatives **38** with sodium cyanide results in phenylacetonitriles. Their subsequent hydrolysis and esterification yield esters of the corresponding phenylacetic acids.[128]



38

X = H, Hal, OAr;
(*a*) NaCN, EtOH, H_2O; (*b*) HCl; (*c*) MeOH.

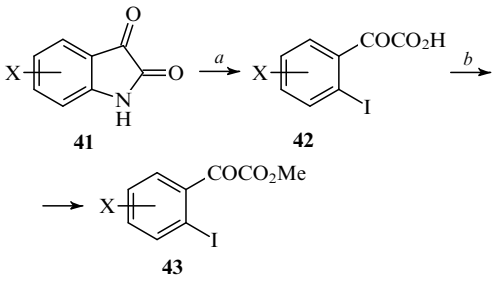
Esters of glyoxylic acids **40** were obtained by oxidation of alkyl mandelates **39** with sodium hypochlorite.[129]



39 **40**

X = Alk, Ar, Het.

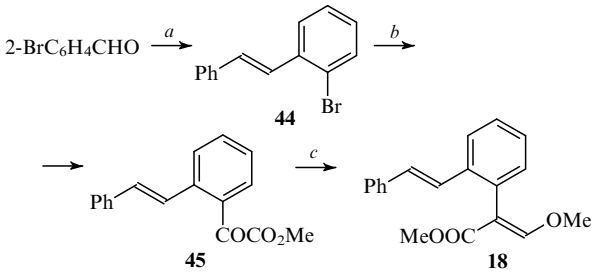
Alternatively, they are synthesised from isatins **41**.[130] To this end, substituted isatins are hydrolysed with a solution of NaOH on heating; the amino group is then substituted for iodine by treatment of the corresponding diazo compound with potassium iodide and copper bronze. The resulting substituted *o*-iodophenylglyoxylic acids **42** are esterified with methyl chloroformate in the presence of triethylamine. In compounds **43**, iodine can be exchanged for other substituents to give a wide range of substituted glyoxylic acids.



41 **42** **43**

(*a*) 1. aq. NaOH; 2. $NaNO_2$, H_2SO_4, H_2O; 3. KI, Cu;
(*b*) $ClCO_2Me$, Et_3N, CH_2Cl_2, 10°C;
X = H, Alk, AlkO, Hal, O_2N, NC.

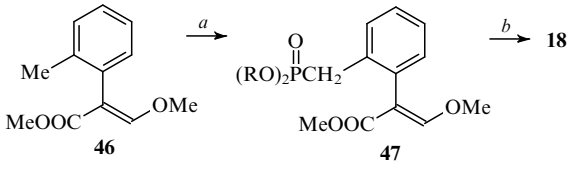
Synthesis of compounds of the type **31** or **36** was also carried out by other methods.[65, 74, 131–133] Thus MOA-stilbene **18** was synthesised from *o*-bromobenzaldehyde. This was coupled with benzylmagnesium bromide. The resulting alcohol was dehydrated in the presence of an acid to give 2-bromostilbene (**44**). The Grignard reagent prepared from the latter was slowly added to an excess of dimethyl oxalate. The ester arylglyoxylic acid **45** that formed was introduced into the Wittig reaction with (methoxymethylene)triphenylphosphorane. The target product **18** contained a small admixture of the *Z*,*E*-isomer.[57]



44 **45** **18**

(*a*) 1. $PhCH_2MgCl$; 2. aq. HCl; 3. H_3PO_4, Δ;
(*b*) 1. Mg; 2. $(CO_2Me)_2$; (*c*) $MeOCH_2P^+Ph_3Cl^-$, NaH.

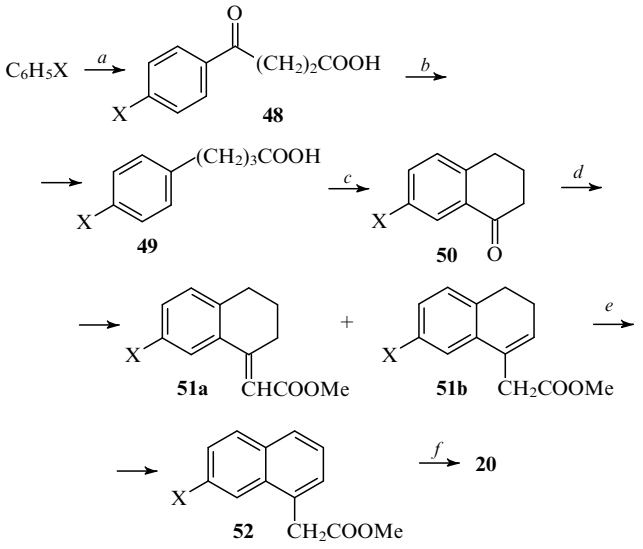
Yet another method to obtain compound **18** is the bromination of (*E*)-2-(2-methylphenyl)-β-methoxyacrylate (**46**) with *N*-bromosuccinimide. The reaction of the resulting bromomethyl derivative with trialkyl phosphite and subsequent condensation of methoxyacrylate **47** with benzaldehyde by the Wittig–Horner–Emmons reaction results in stilbene **18**.[70]

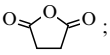


46 **47**

(*a*) 1. NBS; 2. $(RO)_3P$; (*b*) NaH, PhCHO.

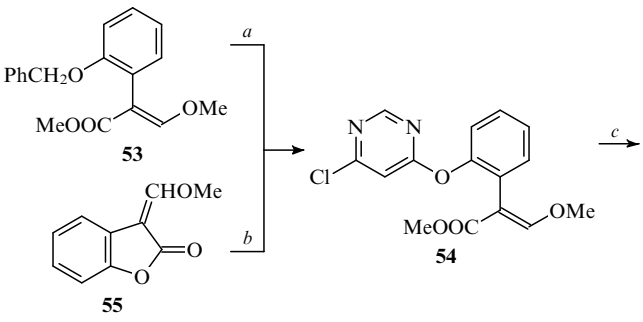
7-Substituted 1-naphthylacrylates **20** that are promising fungicides are synthesised in several steps from substituted benzenes.[131, 134–136]

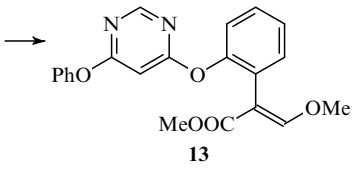


(*a*) $AlCl_3$,  ; (*b*) 1. EtOH; 2. CF_3CO_2H, Et_3SiH; 3. KOH, aq. EtOH; 4. HCl; (*c*) 1. $SOCl_2$; 2. CF_3SO_3H; (*d*) $BrCH_2CO_2Me$, Zn, THF; (*e*) S, 220°C; (*f*) 1. HCO_2Et, NaH; 2. Me_2SO_4; X = H, Ph, Hal, Alk.

First, benzene or its derivative is condensed with succinic anhydride under conditions of the Friedel–Crafts reaction. The 4′-substituted 4-oxo-4-phenylbutyric acid **48** thus obtained is esterified and the keto group is reduced. After hydrolysis of the ester, 4-phenylbutyric acid **49** is converted into an acid chloride, which is further cyclised into a 7-substituted α-tetralone **50**. Its condensation with methyl bromoacetate in the presence of zinc gives a mixture of isomeric methyl esters **51a**,**b**, which is dehydrogenated with sulphur on heating to give 7-substituted methyl naphthylacetates **52**. The latter are converted into the target compounds **20** by condensation with ethyl formate followed by methylation with dimethyl sulfate.

It was proposed to synthesise compound **13** from methyl α-(*o*-benzyloxyphenyl)-β-methoxyacrylate **53**,[137] which is first hydrogenated to remove the protective benzyl group. The resulting phenol is introduced into the reaction with 4,6-dichloropyrimidine in the presence of potassium carbonate to give compound **54**. An alternative procedure is as follows: 3-methoxymethylenebenzo-2-(3*H*)-furanone (**55**)[132] is introduced into reaction with 4,6-dichloropyrimidine in the presence of sodium methoxide. The chlorine atom in pyrimidine **54** synthesised by either of these methods is replaced by heating with 2-cyanophenol in DMF in the presence of potassium carbonate to give compound **13**.

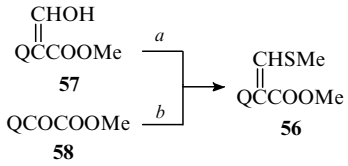




(*a*) 1. H_2, Pd/C; 2. 4,6-dichloropyrimidine, K_2CO_3, DMF; (*b*) NaOMe, 4,6-dichloropyrimidine; (*c*) 2-NCC_6H_4OH, K_2CO_3, DMF.

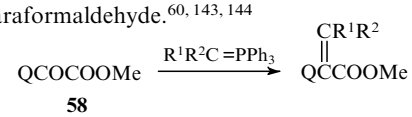
Since the synthesis of β-methoxacrylates according to Schemes 1 and 2 usually gives a mixture of *E*- and *Z*-isomers, it was proposed to use isomerisation of the double bond by treatment with gaseous HCl, $Hg(OAc)_2$,[138] some radical reagents (halogens, *N*-halosuccinimide),[139] various O-, S-, C-, Hal-, or N-nucleophiles [140] to yield (*E*)-β-methoxyacrylates possessing higher biological activity.

Methylthioacrylates **56** can be obtained from the corresponding β-hydroxyacrylates **57**, *e.g.*, *via* tosyl derivatives, which are introduced in the reaction with NaSMe.[141] It was also proposed to synthesise methylthioacrylates **56** from esters of glyoxalic acids **58** by the Wittig reaction, *i.e.*, by analogy with the synthesis of methoxyacrylates.[133]

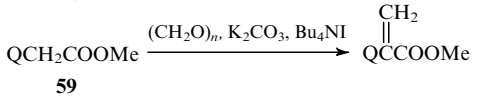


(*a*) 1. TsCl; 2. NaSMe; (*b*) $MeSCH_2P^+Ph_3Cl^-$, Bu^tOK.

Esters of acrylic and crotonic acids and their homologues are obtained in a similar way from esters of glyoxalic acids **58** by the Wittig reaction [60, 61, 142, 143] or by condensation with paraformaldehyde.[60, 143, 144]



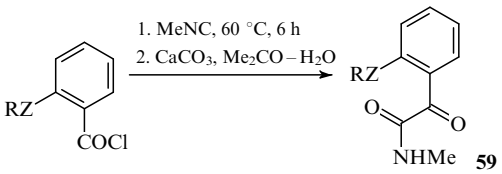
R^1, R^2 = H, Alk.



Esters and amides of methoxyaminoacetic acids are prepared by the reaction of *O*-methylhydroxylamine with the corresponding esters or amides of glyoxylic acid.[62, 63, 145] The target *E*-isomer is formed from a mixture of isomeric esters in the presence of acids.[146]

Similarly, alkylhydrazones of glyoxylates are obtained from alkylhydrazines.[65]

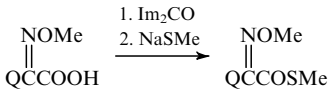
It was proposed to synthesise glyoxylic and methoxyiminoacetic acid amides by aminolysis of the corresponding acid chlorides,[147–149] esters,[97, 150–153] or nitriles (with subsequent deamination of the amidines formed with nitric acid).[154] The synthesis of *N*-methylamides of substituted phenylglyoxylic acids **59** has been patented.[155] The latter are used as intermediates in the synthesis of *N*-methylamides of methoxyiminoacetic acids. This method consists in the reaction of substituted benzoyl chlorides with methylisocyanide followed by hydrolysis of the product formed.

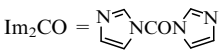


59

R = H, Alk, Hal, Ar, Het; Z = CH_2, O, S, CHOH, CO, CH_2CH_2, CH = CH.

Some *S*-methylmethoxyiminothioacetates that are sometimes more active in trials than compound **14** can be obtained by hydrolysis of methoxyiminoacetic acid esters and subsequent reaction of free acids with sodium methanethiolate[156] in the presence of carbonyldiimidazole.





Methyl *O*-esters and amides of methoxyiminothioacetic acids are obtained by the reaction of esters and amides of metoxyiminoacetic acids with Lawesson's reagent.[157–159]

VI. Conclusion

The modern approach to the synthesis of pesticides that makes use if the natural compounds for identification of biological targets and for modelling new biologically active substances is well known and has often been employed, *e.g.*, for elaboration of pyrethroid insecticides, nereistoxin analogues, juvenoids, neonicotinoids, 4-hydroxycoumarine derivatives, *etc*. Strobilurin analogues also provide an illustrative example of how bioisosterism can be applied for directed enhancement of properties of natural compounds. Despite high biological and particularly fungicidal activity of methoxyacrylate-type antibiotics, their application for plant protection is impeded due to their high sensitivity to light. Nevertheless, it is on the basis of the natural methoxyacrylates that synthetic agrochemical preparations with a basically new mechanism of action have been obtained.

Analogues of the natural MOA-inhibitors have indisputable advantages over other systemic fungicides because of the lack of natural resistant microbial strains. Thus, ICI-A5504 (compound **13**) efficiently inhibits fungi that are resistant to inhibitors of C-14-demethylase, phenylamides, dicarboxyimides, and benzimidazoles.[80]

Strobilurins and their analogues constitute a large group of compounds that are hardly inferior to triazole fungicides in structural diversity. They represent a new class of plant-protecting agents that meet all the demands that are made nowadays for pesticides. Intensive studies aimed at a search for novel biologically active pesticides are currently under way by different manufacturers. However, these studies are still in their infancy and so far only three fungicides have been produced by ICI, BASF, and Shionogi. Probably, original products will be offered very soon by Bayer and Roussel UCLAF.

Interest in this group of compounds is increasing with every passing year as can be evidenced from the number of patent applications. The place occupied by strobilurin analogues on the world pesticide market will be evident in due course.

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