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Synthesis and Herbicidal Activity of Isoxazoledicarboxylic Acid Derivatives*

Peter Münster,‡ Wolfgang Freund, Volker Maywald, Thomas Kükenhöhner, Matthias Gerber, Klaus Grossmann & Helmut Walter

BASF Aktiengesellschaft, D-67056 Ludwigshafen, Germany

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Abstract: Starting from bulk chemicals, novel isoxazoledicarboxylic acid esters were prepared and converted to the corresponding dicarboxylic acid monoamides in a few reaction steps and in high overall yields. Key intermediates for the cycloaddition step were chlorooximinoacetates or amides as nitrile oxide equivalents, prepared in one-pot reactions from diketene or acetoacetic acid esters. Isoxazoledicarboxylic acid monoamides combined good herbicidal activity with chemical flexibility. They were characterized as inhibitors of photosynthesis. Particularly, they affected the photosynthetic electron transport in photosystem II and the rate of carbon dioxide assimilation. Applied post-emergence, the new compounds were found to control a broad spectrum of key weeds in corn. Excellent activity was found on *Abutilon theophrasti* (L.) Medik., *Amaranthus retroflexus* L., *Chenopodium album* L., *Ipomoea spp.*, *Polygonum persicaria* L., *Solanum nigrum* L. and *Xanthium strumarium* L. with rates between 0.2 and 0.5 kg ha^{-1}. As a side-effect, the compounds also showed activity against grass weeds. The compounds are excellent tank-mix partners, e.g. for sulfonylureas, to complete the weed spectrum (*Chenopodium album* L., *Solanum nigrum* L.) and/or to reduce the risk of developing herbicide-resistant weeds.

Key words: isoxazoledicarboxylic acid mono amides, inhibition of photosynthesis

1 INTRODUCTION

Early structural variations of herbicidal imidazolinones and their open-chained precursors led to monoamides of heterocyclic dicarboxylic acids (Fig. 1). Among many different heterocyclic systems synthesized, isoxazole amide derivatives were found to be the most active. These new compounds are inhibitors of photosynthesis. In particular, the inhibitory effects of the herbicides on the Hill reaction of isolated thylakoids from wheat and on the rate of carbon dioxide assimilation in young plants of *Chenopodium album* L. were determined. The isoxazoledicarboxylic acid derivatives show strong herbicidal activity after post-emergence application against a wide range of dicotyledonous weeds, while maize (corn) is tolerant to them. Structure–activity relationships showed that the herbicidal activity depends greatly on the position of the amide group attached to the isoxazole ring.

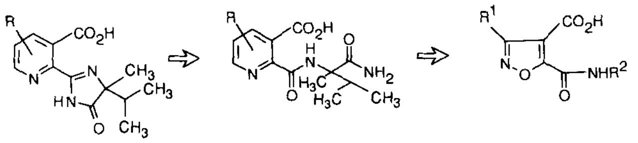

Fig. 1. Retrospective view of finding the isoxazole amide lead structure.

In order to find out the chemical flexibility of this new class of compounds, the substituents R^1 and R^2 were broadly varied, and all regioisomers of isoxazoles with vicinal amide and carboxyl functions were synthesized. Of these, the 3- and 5-amides turned out to be

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[‡] To whom correspondence should be addressed.

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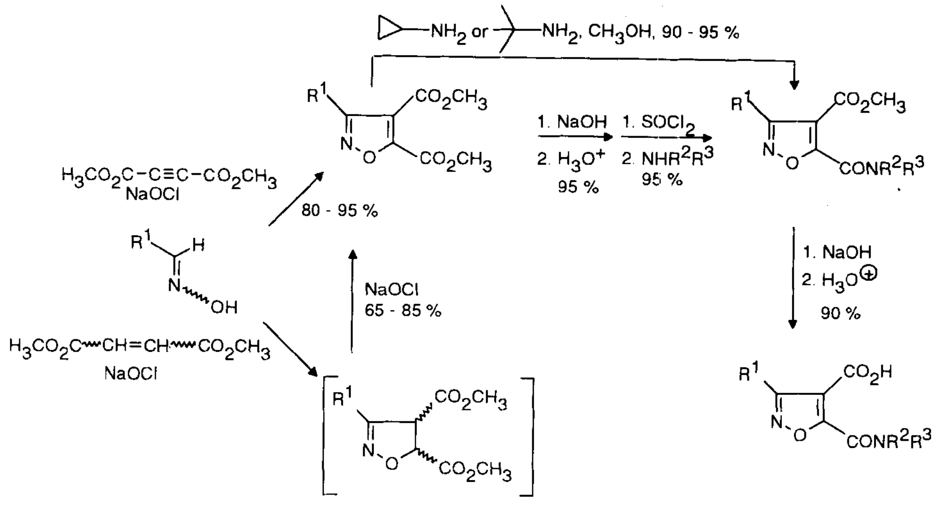


Fig. 2. Synthetic routes to 4-carboxyisoxazole-5-carboxamides.

biologically most relevant and initiated new routes for synthesis.

2 MATERIALS AND METHODS

2.1 Synthesis

2.1.1 4-Carboxyisoxazole-5-carboxamides

5-Carboxamides are synthesized by cycloaddition of nitrile oxides with acetylenedicarboxylic acid esters, selective hydrolysis of the 5-ester and conventional amidation of the intermediate acid chloride (Fig. 2). A more direct and regioselective amidation of the 5-ester is possible in the case of cyclopropyl- and *tert*-butylamine. For industrial purposes, key intermediates like methyl 3-isopropylisoxazole-4,5-dicarboxylate can also be manufactured starting from cheap and readily available maleic or fumaric acid esters instead of acetylenedicarboxylic acid esters.

2.1.2 4-Carboxyisoxazole-3-carboxamides

A very efficient synthesis starts from 2-chloro-2-hydroxyiminoacetamides as nitrile oxide equivalents and *β*-ketoesters (Fig. 3). The nitrile oxide precursors are available by a one-pot reaction of diketene with amines, subsequent nitrosation of the activated methylene group and removal of the acetyl group by chlorination.

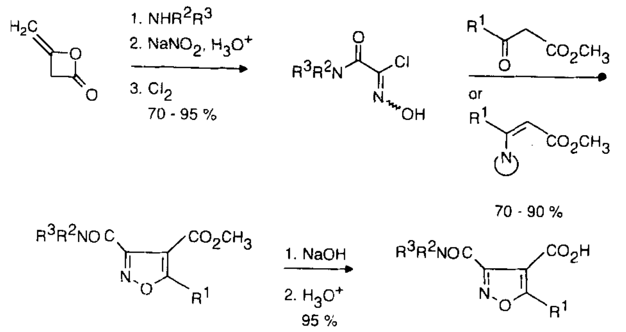


Fig. 3. Synthetic routes to 4-carboxyisoxazole-3-carboxamides.

2.1.3 3-Carboxyisoxazole-4-carboxamides

Analogous to the synthesis of 4-carboxyisoxazole-3-carboxamides, the 3-carboxyisoxazole-4-carboxamides can be prepared by reaction of 2-chloro-2-hydroxyiminoacetates with *β*-ketoamides (Fig. 4).

2.1.4 5-Carboxyisoxazole-4-carboxamides

The starting materials are isoxazole-4-carboxylic acid esters with a protected 5-carboxyl group leading, after a

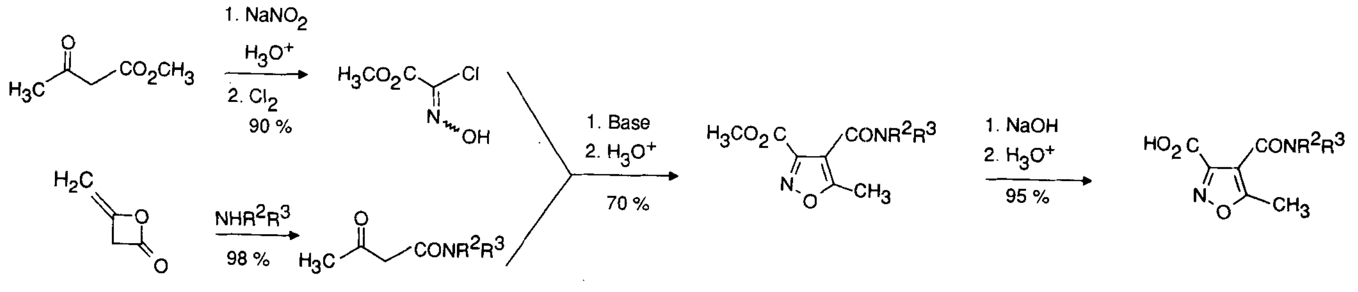


Fig. 4. Synthetic route to 3-carboxyisoxazole-4-carboxamides.

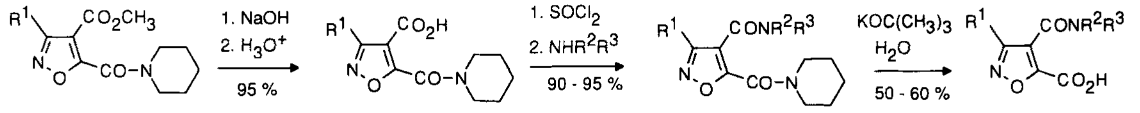


Fig. 5. Synthetic route to 5-carboxyisoxazole-4-carboxamides.

series of saponification and amidation steps, to the 4-carboxamides (Fig. 5).

2.2 Biological tests

2.2.1 Hill reaction

Thylakoids were isolated from wheat (*Triticum aestivum* L. cv. Kanzler) as described by Böger[1] with modifications according to Retzlaff (pers. comm.). Leaf sections (60 g) of young plants grown under greenhouse conditions were homogenized in a medium (200 ml) containing sucrose 0·4 M. tricine-sodium hydroxide buffer (pH 8·0) 50, sodium chloride 10 and magnesium chloride 5 mM. After filtration through surgical gauze and centrifugation, the pellet was suspended in a sucrose-free medium (7 ml).

After additional centrifugation, the pellet was suspended in reaction medium (4 ml) containing sucrose 0·1 M, tricine-HCl (pH 4·0) 50, magnesium chloride 5 mM and chlorophyll 35 $\mu g\ ml^{-1}$. The influence of the compounds on photosynthetic electron transport in photosystem II was performed according to the method of Avron and Shavit.[2] The assay mixture included thylakoid suspension (2·3 ml), test compound) dissolved in 25% methanol, 0·5 ml), and ferricyanide (0·2 ml). During the subsequent illumination, ferrocyanide was formed in the Hill reaction. Then, in darkness, the ferrocyanide was allowed to react with a ferric salt to form the ferrous salt which produced a complex with phenanthroline. The complex was measured photometrically at 510 nm.

2.2.2 Gas-exchange measurements

Gas-exchange measurements for the rate of carbon dioxide uptake as a parameter for carbon dioxide assimilation were made with young plants of *Chenopodium album* L. at the second-leaf stage, as described by Retzlaff and Hamm[3] with modifications noted by Walter *et al.*[4] Eight plants were cultivated hydroponically in a glass chamber (illumination of Osram HQL-R 400 W lamps, *c.* 700 $\mu mol\ m^{-2}\ s^{-1}$, at 25°C) which received a constant stream of air. After one day of adaptation, groups of plants were sprayed uniformly with 0·6 ml per chamber of an aqueous solution of the test herbicide (2 parallel glass chambers). Stock solutions of the compounds were prepared with methanol. Before use, these solutions were diluted with water to the test concentration (final concentration of methanol: 1 ml $litre^{-1}$). In controls, an aqueous solution containing the corresponding methanol concentration was applied. The amount of carbon dioxide assimilated per unit of time was determined continuously from the differences between the carbon dioxide contents in the inflow and outflow stream of air (CO_2 gas analyzer BINOS-IR-VIS/UV, Rosemount, Hanau, Germany).

2.2.3 Greenhouse experiments

All the data were taken from screening-type trials in the greenhouse.[5] The vessels employed were plastic flower pots having a volume of 300 cm^3. The substrate was a sandy loam with 80% sand, 10% silt, 10% clay, 3·3% organic matter, a waterholding capacity of 32%, and pH 6·5.

Treatments were made post-emergence at various growth stages in order to determine the application window. All doses are given in kg ha^{-1} of active ingredient. Assessments of herbicidal activity and selectivity were made at two and four weeks after treatment on a scale 0 to 100, 0 denoting no damage and 100 denoting complete destruction of at least the visible plant parts.

The following plant species were used under greenhouse conditions: *Amaranthus retroflexus* L. (redroot pigweed), *Chenopodium album* L. (farhen); *I pomoea spp* (morning glory), *Polygonum persicaria* L. (redshank), *Solanum nigrum* L. (black nightshade) and *Zea mays* L. (maize).

2.2.4 Field trials

Field trials were carried out at the BASF research farms in Brazil, South Africa, Spain and the USA with post-emergence treatments at various growth stages of the crops and weeds. Trial layout and evaluations followed standard procedures.

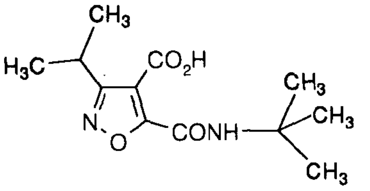
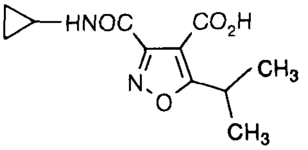
3 RESULTS AND DISCUSSION

3.1 Mode of action

As shown in Table 1, this new type of herbicide effectively inhibited the Hill reaction of isolated thylakoids from wheat with ferricyanide as electron acceptor. The inhibitory potency of the 3-amide was similar to that of the herbicide diuron, a well-known inhibitor of the photosynthetic electron flow,[6] and surpassed the activity of the 5-amide. This is in accordance with the difference in the herbicidal potential of both compounds observed in the greenhouse experiments (Fig. 6). Concomitantly, the compounds were able to reduce rapidly and considerably the rate of carbon dioxide assimilation in C. album plants after foliar treatment (Table 2). It is concluded that the herbicides primarily influence the

TABLE 1

Inhibitory Effects *in vitro* of Isoxazole carboxamides and of diuron on the Photosynthetic Electron Transport in Photosystem II (Hill Reaction) using Isolated Thylakoids from Wheat

Compound	*Concentration* (*μM*)	*Inhibition* (%)
	100 10 1	78 31 21
	100 10 1	94 66 58
Diuron	1	63

photosynthetic electron transport in photosystem II, which results in an inhibition of carbon dioxide assimilation.

3.2 Structure–activity relationships (SAR)

The biological screening of hundreds of different isoxazoledicarboxylic acid derivatives demonstrated that the nature and the position of the amide function on the isoxazole ring is mainly responsible for influencing the activity (Fig. 6). In the case of the most active 5-amides and 3-amides, the nature of the alkyl substituents influenced the selectivity against corn.

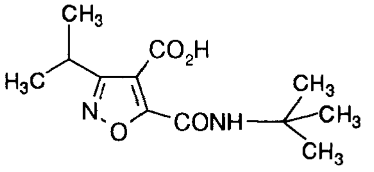
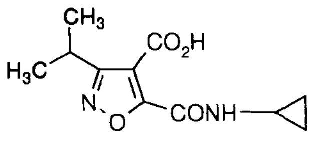
3.3 Field trials

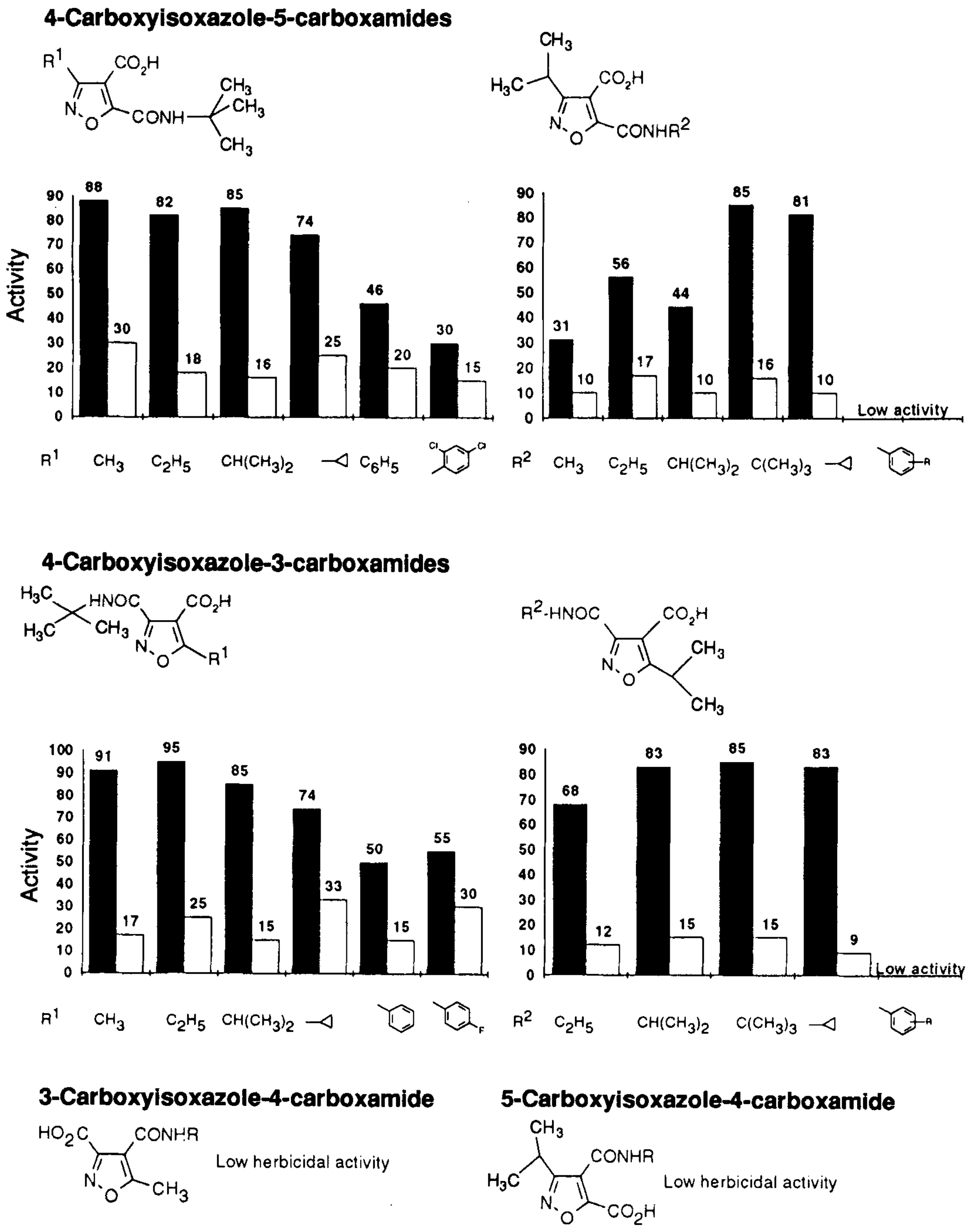
3.3.1 Weed spectrum and selectivity

The new isoxazoledicarboxylic acid derivatives applied post-emergence at rates between 0·2 and 0·5 kg ha^{-1} controlled a broad spectrum of significant weed species

TABLE 2

Inhibitory Effects *in vitro* of Isoxazole carboxamides on the Assimilation of Carbon Dioxide in *Chenopodium album* Plants 24 h after Foliar Treatment

Compounds	*Concentration* ($g \times ha^{-1}$)	*Inhibition* (% *of control*)
	1000 500 250	94 84 71
	1000 500 250	98 77 67



4-Carboxyisoxazole-5-carboxamides

4-Carboxyisoxazole-3-carboxamides

3-Carboxyisoxazole-4-carboxamide

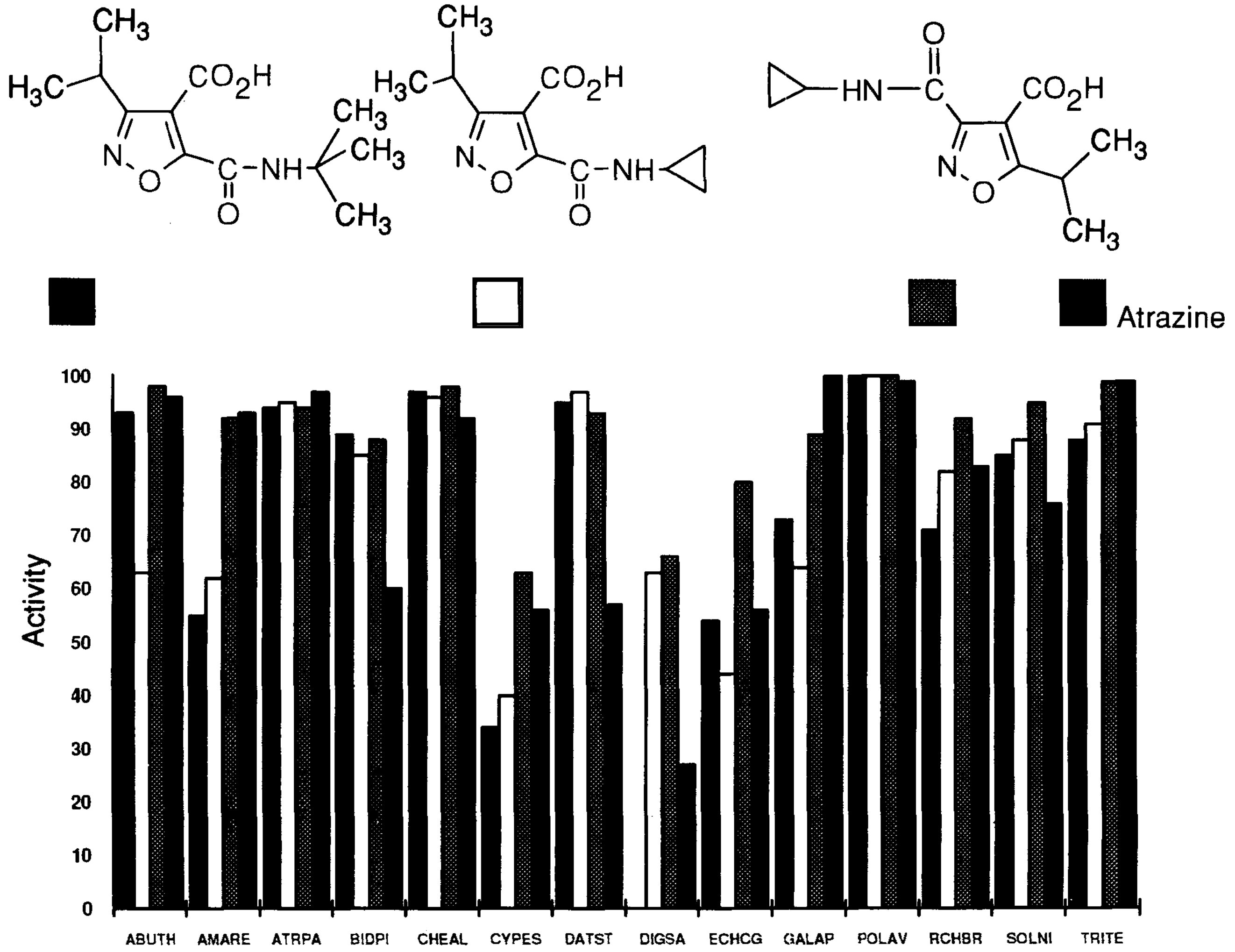
5-Carboxyisoxazole-4-carboxamide

Fig. 6. Structure–activity relationships for carboxyisoxazole-carboxamides. Activity against (■) five test plants listed in Section 2.2.3 (mean value), (□) maize, when applied at 0·5 kg ha^{-1}. 0 = no damage, 100 = complete destruction of aerial parts.

together with excellent selectivity in corn. From indigenous trials in the 1990 and 1991 growing seasons, the new compounds demonstrated weed control superior to atrazine, based on per-unit activity. Excellent control of velvetleaf, redroot pigweed, *Atriplex patula* L. (spreading orache), Farhen and black nightshade was achieved with 0·5 kg ha^{-1} (Fig. 7). Furthermore, excellent activity was also found on the following weed species: *Abutilon theophrast*; (L.) Medik., *Amaranthus albus* L., *A. hybridus* L., *A. spinosus* L., *Ambrosia eliator* L., *Sinapis arvensis* L., *Datura stramonium* L., *Heliotropium europaeum* L., *Matricaria inodora* L., *Polygonum aviculare* L., *P. persicaria* L., *Tagetes minuta* L., *Tribulus terrestris* L. and *Xanthium strumarium* L.

Good activity was found on: *Bidens pilosa* L., *Commelina benghalensis* L., *Galium aparine* L. and *Ipomoea obscura* L. In addition, good side effects were observed with rates of 0·75 kg ha^{-1} on the following grass weeds and sedges: *Digitaria adscendens* Henr., *Echinochloa crus-galli* Beauv., *Richardia brasiliensis* (Moq.) Gomez, *Setaria viridis* Beauv. and *Cyperus esculentus* L.

As with bentazone, the new isoxazole derivatives



number of trials	84	93	95	31	99	18	30	9	15	21	6	22	12	15	■
	63	78	3	31	69	15	26	3	6	18	6	18	12	15	□
	72	90	3	38	80	36	36	21	27	15	6	36	15	18	▨
	30	64	12	5	120	10	6	25	58	51	16	6	25	6	■

Fig. 7. Herbicidal activity of three isoxazole amides (0·5 kg ha^{-1} + oil concentrate) compared with that of atrazine at 1 kg ha^{-1} + oil concentrate. Test weeds are described in Sections 2.2.3 and 3.3.1.

reached the highest activity levels under warm and good growing conditions. Under field conditions, no activity in soil or carry-over potential was found.

3.3.2 Application window and tank-mixes

The new isoxazoledicarboxylic acid derivatives are post-emergence herbicides with excellent selectivity in corn, best herbicidal activity being obtained up to the three- to four-leaf stage of the weeds. Additives improve and stabilize the activity of the compounds. The isoxazoles are excellent tank-mix partners with other herbicides in corn, including acetolactate synthase (ALS-)inhibitors. One example is presented in Fig. 8 with nicosulfuron as the partner.

In comparison to the activity of the single compounds, the tank-mix reached a very high activity level across the weed spectrum tested (Fig. 8). Excellent complementary effects to nicosulfuron were achieved on *Ab. theophrash*; *Am. eliator*, *C. album*, *Piariculare*, *P. convulvulus* and *S. nigrum*. Where the two compounds overlap in the weed spectrum, the new isoxazole compounds are suitable as herbicides to reduce the risk of developing ALS-resistant weeds in corn.

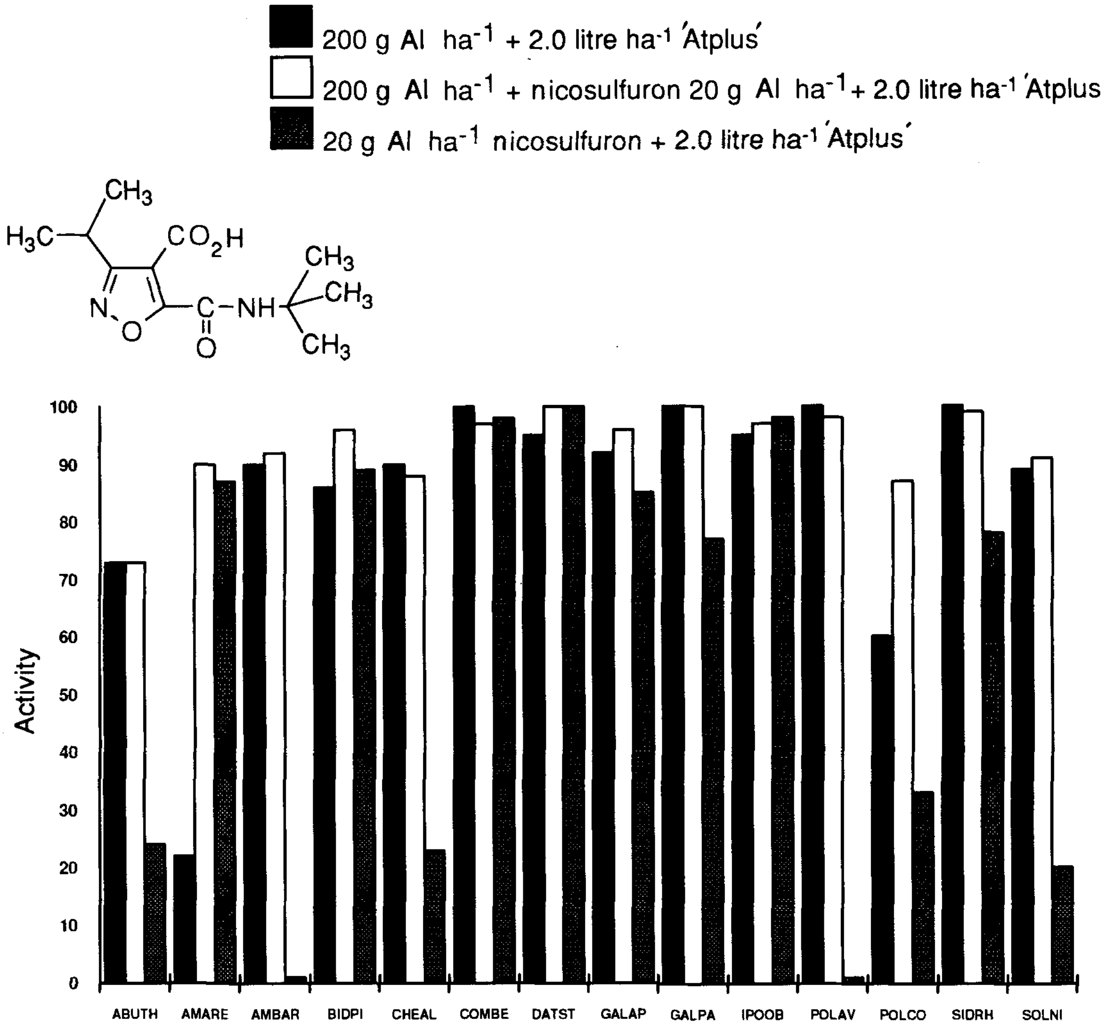


Fig. 8. Selectivity and herbicidal activity of an isoxazole carboxamide and nicosulfuron combination in corn. AMBAR = *Ambrosia astemesiifolia* L., GALPA = *Galium palustre* L., SIDRH = *Sidu rhombifolia* L., other test weeds are described in Section 3.3.1 or 3.3.2.

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