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Synthesis of new plant growth regulator: *N*-(Fatty acid) *O*-aryloxyacetyl ethanolamine

Liang Han,[a] Jian-Rong Gao,[a] Zheng-Ming Li,[b,*] Yun Zhang[c] and Wei-Ming Guo[c]

[a]*College of Chemical Engineering and Material Science, Zhejiang University of Technology, Hangzhou 310014, China*
[b]*National Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, China*
[c]*College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China*

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Abstract—*N*-(Fatty acyl) *O*-aryloxyacetyl ethanolamines, prepared from *N*-acylethanolamine (NAE) and aryloxyacetic acid, were tested for plant growth regulating activity. Compared with *N*-stearoylethanolamine, most compounds exhibit improved plant growth stimulating activity. In particular, those with chlorine on aryl ring show better activity than 2,4-dichlorophenyloxyacetic acid in stimulating hypocotyls elongation of rape which indicates that chlorine on aryl ring appears significant. Moreover, these derivatives display improved solubility.

N-Acylethanolamines (NAEs), a family of endogenous fatty acid amides, are known in mammals since some decades. They are proved to be an important bioactive substance in microgram level existing in the animal cells and play biochemical and pharmacological role.[1] However, their widespread occurrence, metabolism, and physiological significance in plants have only recently begun to be appreciated.[2] More investigation supported the fact that NAEs play a lipid mediator role in plants and are implicated as transducers in plant defense signaling,[2d,f,3] and at elevated levels, interfered with normal seedling root development.[4] The identification and active metabolism of NAEs in these physiological situations supports the emerging concept that NAEs are endogenous signaling compounds in plant systems.[5] Furthermore, LPE(lysophosphatidylethanolamine), capable of inhibiting plant PLD α (phospholipase α), has a profound effect on the physiological symptoms associated with postharvest senescence of flowers and fruits[6]; however, NAE12:0 and NAE14:0 are at least a 100-fold more potent than LPE in inhibiting PLD α activity, so these NAEs may found novel agrochemical applications in the future.[7,8] The applications of exogenous NAEs further confirmed the recognition of their important role in plant.[2f,9]

Though the above reports are exciting, NAEs have very low solubility in water and limited solubility in most common organic solvents, which discourages further study and application. It urges the molecular modification of NAEs to improve the solubility, which has not been reported to date. On the other hand, it is well known that substituted aryloxyacetic acids exhibit high plant growth regulating activity.[10] Yet, sometimes they induce unfavorable responses. For example, when substituted aryloxyacetic acids were used to improve fruit set, some suppression of leaf growth happened.[10] The report that the ester of aryloxyacetic acids can not only improve the translocatibility but also reduce the extent of side effects encourages us to incorporate the aryloxyacetic acid type regulator into NAEs. A series of *N*-(fatty acyl) *O*-aryloxyacetyl ethanolamines were synthesized and their bioactivity is reported here. The preparation of title compounds followed the reaction sequence depicted in Scheme 1.

Several NAE types have been identified in a variety of plant species. These NAE types identified contain acyl chains of 12-18C in length and up to three double bonds, reflecting the typical acyl moieties prevalent in higher plants.[2a] In terms of bioactivity, plants are particularly responsive to low concentration of medium chain

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* Corresponding author. Tel.: +86 0 22 23503732; fax: +86 0 22 23505948; e-mail: nkzml@vip.163.com

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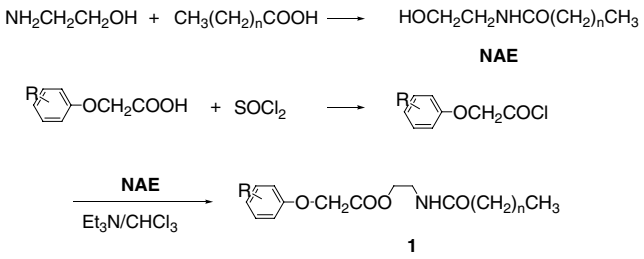

Scheme 1.

saturated NAEs; whereas animal physiology is largely regulated by low concentration of long chain polyunsaturated NAEs (e.g., NAE 20:4).[5] In our work, four kinds of NAEs, namely NAE12:0, NAE14:0, NAE16:0, NAE18:0, were synthesized to incorporate into the structure of several aryloxyacetic acids. NAE18:0 was chosen to couple with five kinds of aryloxyacetic acids to study the influence of substituent group on bioactivity. And the 2,4-dichlorophenoxyacetic acid (2,4-D) was used to react with all synthesized NAEs to investigate the bioactivity variety along with chain length.

NAEs were prepared by refluxing 1 mol of the fatty acid with 1.5 mol of ethanolamine for 6 h in 60–90% yield.[11] The products were purified by recrystallization from the solution of trichloromethane and acetone instead of 95% ethanol of Ref. 11. Aryloxyacetic acids were prepared according to Ref. 12 and then transformed to the acid chlorides by refluxing with $SOCl_2$ for 5 h. NAEs, suspended in $CHCl_3$ in the presence of Et_3N, were dissolved gradually with the addition of the acid chlorides, which indicated the reaction happened and the products had better solubility in $CHCl_3$ than NAEs. After the usual workup, title compounds **1** were obtained through the purification by column chromatography. Structures of title compounds were confirmed by elemental analyses and ^1H NMR.[13]

Table 1. Solubility of some title compounds and parent NAEs

Compound	*n*	R	Solubility (g)		
			$CHCl_3$	EtOAc	EtOH
NAE12:0	10	—	1.76	0.38	9.14
1j	10	2,4-2Cl	15.10	11.0	10.42
NAE18:0	16	—	0.16	0.03	0.34
1a	16	*m*-CH_3O	7.76	2.67	1.99
1b	16	*o*-CH_3	53.97	8.27	3.08
1e	16	*p*-Cl	4.37	1.54	1.88
1g	16	2,4-2Cl	10.22	3.54	1.03

On the other hand, the solubility of title compounds and NAEs was detected in $CHCl_3$, EtOAc, and EtOH. The solubility data of some title compounds with NAE12:0 and NAE18:0 are listed in Table 1. As compared with respective NAE, title compounds show evident solubility improvement.

Since NAE was reported to prolong the shelf life of cut flowers, delay deterioration and leaf drop, and extend the overall appearance and quality of the plant cutting,[9] title compounds were tested for their plant growth stimulating activity in hypocotyls elongation of rape, cotyledon expansion of cucumber, and coleoptiles growth of common wheat.[14–16] The increase percent in these assays over control samples with no regulator is listed in Table 2 and also are the data of NAE18:0 and the conventional plant growth regulator 2,4-D for comparison. Just like NAE18:0 and 2,4-D, title compounds exhibit good stimulating effect on hypocotyls elongation of rape. In hypocotyls elongation test, improved stimulating effect was observed for all title compounds except compound **1a** when compared with NAE18:0, and compounds **1e**–**1j** with different chain length and substituent group even have better growth stimulating activity than 2,4-D. It is interesting to note that the hypocotyls elongation of rape appears strongly associated with the substituent group on the benzene ring. The compounds with substituent chlorine are obviously superior to those with substituent methyl and methoxy group, which suggests that chlorine on aryl ring appears significant. For example, compound **1g** has an increase percent of 73.8% in stimulating hypocotyls elongation of rape, while the increase percent of compound **1b** is only 57.0%. However, introduction of additional chlorine does not affect the elongation of rape hypocotyls and the activity of 4-chloro-substituent **1e** is comparable to that of 2,4-dichloro-substituent **1g**. In coleoptiles growth test of common wheat, all title compounds also exhibit better stimulating effect than NAE18:0 except compound **1b**,

Table 2. Plant growth regulating bioactivity in vitro

Compound	*n*	R	Increased bioactivity (%, 10 mg/L)		
			Hypocotyls elongation of rape	Cotyledon expansion of cucumber	Coleoptiles growth of common wheat
1a	16	*m*-CH_3O	−1.4	−0.1	5.0
1b	16	*o*-CH_3	57.0	5.7	0.7
1c	16	*p*-CH_3	38.7	9.8	3.3
1d	10	*p*-CH_3	54.9	5.9	4.1
1e	16	*p*-Cl	71.8	6.3	5.0
1f	10	*p*-Cl	71.8	2.9	16.0
1g	16	2,4-2Cl	73.8	0.7	7.5
1h	14	2,4-2Cl	67.3	3.5	17.0
1i	12	2,4-2Cl	68.1	−0.1	8.9
1j	10	2,4-2Cl	69.0	4.0	17.9
NAE(18)	16	—	35.3	6.2	2.1
2,4-D	—	2,4-2Cl	65.7	0.8	28.3

unfortunately, only compound **1c** shows a little improved stimulating activity in cotyledon expansion test of cucumber. The change of fatty chain length seems to have intricate effect on the bioactivity. Compound **1f** has the same bioactivity in stimulating hypocotyls elongation of rape as **1e**, though there is a difference of six methylenes in their fatty chain structure. But when stimulating cotyledon expansion of cucumber and coleoptile growth of common wheat, compounds with fatty chain of 16 carbons and 12 carbons have better activity than compounds with fatty chain of 18 carbons and 14 carbons.

In conclusion, a series of *N*-(fatty acyl) *O*-aryloxyacetyl ethanolamines were synthesized by reaction of NAEs of different chain length with substituted aryloxyacetic acids. All new compounds have improved solubility. The preliminary bioactivity data show that most of them exhibit better plant growth stimulating effect than NAE18:0, moreover, those with chlorine on benzene ring have better stimulation of hypocotyls elongation than conventional 2,4-D.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.03.013.

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13. *General method for preparing title compounds.* A mixture of NAE (1 mmol) and Et_3N (1.2 mmol) in dry $CHCl_3$ (5 ml) was stirred at room temperature and then the solution of aryloxyacetic acid chloride (1.2 mmol) in dry $CHCl_3$ (2 ml) was added drop by drop. During the addition, NAE was dissolved gradually and the solution turned yellow. After stirred for 12 h, the solution was washed with water and then dried with $MgSO_4$. The solvent was removed and column chromatography of the residue with PE/EtOAc (1.5:1, v/v) gave the product as a white solid. Compound **1b**, yield 75%: mp 63–64 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.20–7.12 (dd, 2H, Ar<u>H</u>), 6.94–6.90 (t, 1H, Ar<u>H</u>), 6.72–6.69 (d, 1H, Ar<u>H</u>), 5.45 (br s, 1H, N<u>H</u>CO), 4.70 (s, 2H, ArOC<u>H</u>$_2$), 4.28–4.24 (t, 2H, OC<u>H</u>$_2$CH$_2$N), 3.51–3.46 (dd, 2H, OCH$_2$C<u>H</u>$_2$N), 2.30 (s, 3H, ArC<u>H</u>$_3$), 2.08–2.02 (t, 2H, C<u>H</u>$_2$CONH), 1.58–1.53 (t, 2H, C<u>H</u>$_2$CH$_2$CO), 1.25 (br s, 28H, (C<u>H</u>$_2$)$_{14}$), 0.90–0.86 (t, 3H, C<u>H</u>$_3$(CH$_2$)$_{14}$) ppm; Anal. calcd for $C_{29}H_{49}NO_4$: C 73.22, H 10.38, N 2.94; found: C 73.24, H 10.40, N 2.85.
14. *Hypocotyls elongation test of rape.* After soaking, the seeds with similar magnitude were chosen to use. Tested compounds were dissolved in DMF and later dropped evenly on 6-cm-diameter fitter paper. After air-volatilization of solvent, the filter paper was placed in 6-cm-diameter glass utensil with distilled water to give 10 mg/L compound solutions, and then ten seeds were added. The seeds were cultured at 25 °C in dark. The length of hypocotyls was measured after 3 days and compared with those treated with distilled water to estimate the activity. Two replicates were included in the evaluation.
15. *Cotyledon expansion test of cucumber.* After soaking, the seeds were germinated in covered enamelware containing 0.7% agar and cultured for 3 days in dark at 26 °C, and then the cotyledons of similar magnitude were chosen to use. Tested compounds were dissolved in DMF and later dropped evenly on 6-cm-diameter fitter paper. After air-volatilization of solvent, the filter paper was placed in 6-cm-diameter glass utensil with distilled water to give 10 mg/L compound solutions and ten pieces of cotyledons were added. The cotyledons were cultured in light (300Lux, 26 °C). After 3 days, the total weight of cotyledon was measured and compared with those treated with distilled water to estimate the activity. Two replicates were included in the evaluation.

16. *Coleoptiles growth test of common wheat.* After soaking, the seeds were germinated in covered enamelware containing 0.7% agar and cultured for 3 days in dark at 25 °C. When the seedling grew to 2.5–3.0 cm tall, the first 3 mm of coleoptile top was rejected. Coleoptile (5 mm) was truncated and dunked in distilled water for 1 h to remove endogenous hormone, then it was chopped into 10 segments. Tested compounds were dissolved in DMF and later dropped evenly on 6-cm-diameter fitter paper. After air-volatilization of solvent, the filter paper was placed in 10 ml beaker with 0.01 mol/L phosphoric acid-citric acid buffer solution (pH 5) containing 2% sucrose to give 10 mg/L compound solutions and ten coleoptile segments were added afterward. The coleoptiles were cultured at 25 °C in dark for 18–20 h and then the length of coleoptiles was measured and compared with those treated without tested compounds to estimate the activity. Two replicates were included in the evaluation.