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# BIOLOGICAL ACTIVITIES OF SOME *ARGYRANTHEMUM* SPECIES

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**Key Word Index**—*Argyranthemum*; Asteraceae; biological activities; *A. frutescens*; acetylenes; structural elucidation.

**Abstract**—Seven species of the genus *Argyranthemum* were studied for antimicrobial and cytotoxic activities. *Argyranthemum adauctum*, *A. foeniculaceum* and *A. frutescens* showed antimicrobial activity against Gram-positive and Gram-negative bacteria and cytotoxic activity against HeLa and Hep-2 cell lines. Two new acetylenic compounds, frutescinol isovalerate and 3′-demethyl frutescinol isovalerate, were isolated from *A. frutescens* and their structures elucidated by spectroscopic studies. © 1997 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

The genus *Argyranthemum* Webb ex Sch.-Bip. (Compositae, Anthemideae, Chrysantheminae) has had an uncertain taxonomic identity throughout its history [1]. For instance, it has been considered a section of the complex genus *Chrysanthemum* L. and it has been included in five other different genera. Some species are endemic to the Macaronesia (Madeira, Selvagens and Canary archipelagos) [2]. There are *ca* 20 species of *Argyranthemum* in the Canary Islands, some of which, for example *A. adauctum* (Link) Humphr. and *A. frutescens* (L.) Sch.-Bip., are very polymorphic and difficult to analyse systematically.

The genus *Argyranthemum* is a good source of secondary metabolites, such as polyacetylenes, sesquiterpene lactones, aromatic esters [3] and spirostanic ethers [4]. Several chemical, synthetic and taxonomic studies have been reported [4, 5], but there is little information on the biological activities of the secondary metabolites present in *Argyranthemum*.

In the present work, we report the screening for antimicrobial and cytotoxic activities of seven species of the genus *Argyranthemum*. We also report the isolation and structural elucidation of two new acetylenic compounds **1** and **2**, from *A. frutescens*.

## RESULTS AND DISCUSSION

Nineteen crude extracts of different plant parts, from seven species of *Argyranthemum* were assayed for antimicrobial and cytotoxic activities (Tables 1 and 2). Acetone extracts from the roots of *A. foeniculaceum*, *A. Adauctum* and *A. frutescens* were the most active, exhibiting a broad spectrum of activity against Gram-positive and Gram-negative bacteria. All extracts were inactive against the yeasts *Candida albicans* and *Saccharomyces cerevisiae*. With respect to cytotoxic activity, it was concluded that the crude extracts from the roots of *A. frutescens* and *A. foeniculaceum* were the most active against the two cell lines used.

Based on the screening results, an extract of the roots of *A. frutescens* was studied. Two new compounds, frutescinol isovalerate (**1**) and 3′-demethylfrutescinol isovalerate (**2**), and nine known compounds (**3**–**11**), were obtained.

The structures of the new compounds, **1** and **2**, were determined by spectroscopic studies including $^{1}H$–$^{13}C$ heteronuclear correlation (HMQC) and long-range correlation spectra with inverse detection (HMBC) and by correlations with the co-occurring known aromatic esters.

Product **1** was isolated as an optically active oil and its UV spectrum revealed typical diyne chromophore absorption (303.4, 250.8, 242.0 and 237.6 nm). It showed a $[M]^+$ at $m/z$ 342 with a base peak at $m/z$ 257. The NMR data (Tables 3 and 4) indicated that **1** was an aromatic acetylene similar to frutescinol acetate (**4**) with an isovaleryl group instead of an acetyl group at C-1.

The spectral data of compound **2** (Experimental and Tables 3 and 4) indicated the presence of 3′-

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Table 1. Antimicrobial activity of crude acetone extracts of *Argyranthemum* species

| Species | Plant part | Inhibition zones (mm) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | | S.a.[a] | S.e. | S.al. | B.s. | B.p. | B.m. | M.l. | E.c. | K.p. | P.m. | S.t. |
| Ampicillin | | 38 | 32 | 27 | – | 10 | n.a. | 60 | n.a. | n.a. | 25 | n.a. |
| Streptomycin | | 11 | 21 | 25 | 27 | 22 | n.a. | 20 | n.a. | n.a. | 16 | n.a. |
| *A. gracile* | Trunk | 10 | 7.5 | – | – | – | – | – | – | – | – | – |
| (Sch.-Bip.) Humphr. | Root | – | – | – | – | – | – | – | – | – | – | – |
| *A. coronopifolium* | Trunk | 8.5 | – | – | – | – | – | – | – | – | – | – |
| (Willd.) Humphr. | Petal | 9.5 | – | – | – | 10.5 | 10 | – | – | – | – | – |
| *A. hierrense* | Trunk | – | 12 | – | – | – | – | – | – | – | – | – |
| Humphr. | | | | | | | | | | | | |
| *A. broussonetii* | Trunk | 13 | 11 | – | – | – | – | – | – | – | – | – |
| (Pers.) Humphr. | Root | – | – | – | – | – | – | – | – | – | – | – |
| | Petal | 9 | – | – | – | – | – | – | – | – | – | – |
| *A. foeniculaceum* | Root | 23 | 34 | 44 | 17.5 | – | 25.5 | 37 | 13.5 | 11.2 | 13 | 14 |
| (Willd.) Webbex | Aerial | – | 9 | – | – | – | – | – | – | – | – | – |
| Sch.-Bip. | Seeds | 13 | 10 | – | – | – | – | – | – | – | – | – |
| *A. adauctum* | Trunk | 10 | 10.5 | – | – | – | – | – | – | – | – | – |
| (Link) Humphr. | Root | 17 | 31 | 41 | 14 | 7 | 25.5 | 37 | 11.5 | 10.5 | 11 | 13 |
| | Petal | 9.5 | – | – | – | – | 9.5 | – | – | – | – | – |
| | Leaves | 9 | – | 9 | 9 | – | – | – | – | – | – | – |
| *A. frutescens* | Trunk | – | 13 | – | – | – | 12.5 | 18 | – | – | – | – |
| (L.) Sch.-Bip. | Root | 23 | 31 | 32.6 | 20 | – | 20.5 | 36 | 13 | – | 9.5 | 14 |
| | Leaves | – | 12 | – | – | – | 7.5 | – | – | – | – | – |
| | Seeds | – | – | – | 9.5 | – | 7.5 | – | – | – | – | – |

n.a.: not assays.

– No inhibition observed.

[a] Microorganisms: S.a., *Staphyloccocus aureus*; S.E., *S. epidermidis*; S.al., *S. albus*; B.s., *Bacillus subtilis*; B.p., *B. pumilus*; B.m., *B. megaterium*; M.l., *Micrococcus luteus*; E.c., *Escherichia coli*; K.p., *Klebsiella pneumoniae*; P.m., *Proteus mirabilis*; S.t., *Salmonella typhimurium*.

All extracts were inactive against *Pseudomonas aeruginosa* and the yeasts, *Candida albicans* and *Saccharomyces cerevisiae*.

Table 2. Cytotoxic activity of crude extracts from *Argyranthemum* species

| Species | Plant part | $IC_{50}$ ($\mu$g ml$^{-1}$) HeLa | Hep-2 |
|---|---|---|---|
| *A. foeniculaceum* | Root | 49.5 | 68.0 |
| *A. adauctum* | Root | 54.0 | >100 |
| | Leaves | 78.0 | >100 |
| *A. frutescens* | Root | 37.0 | 68.0 |
| | Seeds | 33.4 | >100 |

demethylfrutescinol isovalerate. However, an attempt to determine its stereochemistry using the Horeau method was unsuccessful.

There are no $^{13}$C NMR data in the literature for compounds similar to the aromatic esters obtained from *Argyranthemum*. For this reason we consider it worthwhile to include in this paper $^{13}$C NMR data (Table 4) and also $^{1}$H NMR data with values of coupling constants confirmed by bidimensional experiments (Table 3).

Compounds **2–8** and **10**, were assayed to determine the minimal inhibitory concentration (MIC) against bacteria and yeasts (Table 5). Compound **3** showed significant activity against Gram-positive bacteria and a moderate effect against Gram-negative bacteria. Products **4** and **5** also showed activity against Gram-positive bacteria.

Analysis of the results shown in Table 5 allowed us to establish a structure-activity relationship. Greater antimicrobial activity is related to the presence of an acetyl group C-1 and a hydroxyl group on the aromatic ring in a *meta*-position to the triple-bond chain. Cytotoxic activity of **2–8** and **10** against HeLa and Hep-2 cells is shown in Table 6. Compounds **3** and **5** were active against the two cell lines used, whereas compounds **2**, **4** and **10** were active only against HeLa cells.

## EXPERIMENTAL

*General.* $^{1}$H and $^{13}$C NMR were run in $CDCl_3$ at 200 and 50 MHz, respectively, with TMS as int. standard. HMBC and HMQC were recorded at 400 MHz.

*Plant collection.* Plant material was collected (May 1993) in different places in Tenerife, Canary Islands. Voucher specimens are deposited in the TFC file in the Department of Botany of the University of La Laguna.

*Preparation of crude extracts.* Samples of 1 g of fr. plant material were exhaustively extracted with cold $Me_2CO$. Extracts were concd *in vacuo* and residues dissolved in DMSO at 10 mg ml$^{-1}$.

Table 3. $^1H$ NMR (200 MHz) ($CDCl_3$) of compounds **1–9**

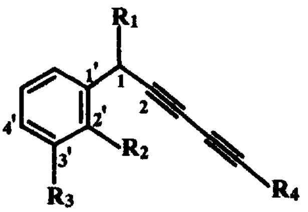
| H | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.58 *s* | 7.18 *s* | 7.15 *s* | 6.57 *s* | 7.28 *s* | 6.48 *s* | 3.94 *s* | — | 6.59 *s* |
| 6 | 1.93 *s* | 1.94 *s* | 1.94 *s* | 1.93 *s* | 1.91 *s* | 1.95 *s* | 1.92 *s* | 2.09 *s* | 2.10 *s* |
| 2′ | — | — | — | — | — | 7.30–7.55 *m* | — | — | — |
| 3′ | — | — | — | — | 7.93 *d* | 7.30–7.55 *m* | — | — | — |
|  |  |  |  |  | (7.8) |  |  |  |  |
| 4′ | 6.96 *dd* | 7.03 *dd* | 7.04 *dd* | 6.95 *dd* | 7.56 *t* | 7.30–7.55 *m* | 7.06 *d* | 7.80 *d* | 6.98 *d* |
|  | (1.0, 8.0) | (1.4, 8.0) | (1.3, 8.0) | (1.2, 8.0) | (7.5) |  | (8.3) | (7.9) | (8.2) |
| 5′ | 7.40 *t* | 7.46 *t* | 7.47 *t* | 7.41 *t* | 7.41 *t* | 7.30–7.55 *m* | 7.38 *t* | 7.50 *t* | 7.44 *t* |
|  | (7.9) | (8.0) | (8.0) | (8.0) | (7.6) |  | (7.8) | (7.9) | (8.0) |
| 6′ | 7.30 *dd* | 7.34 *dd* | 7.35 *dd* | 7.31 *dd* | 7.82 | 7.30–7.55 *m* | 6.93 *d* | 7.76 *d* | 7.31 *d* |
|  | (1.0, 8.0) | (1.4, 8.0) | (1.3, 8.0) | (1.2, 8.0) | (7.8) |  | (8.3) | (7.9) | (8.3) |
| $COOC\underline{H}_3$ | 3.89 *s* | 3.98 *s* | 3.98 *s* | 3.89 *s* | 3.90 *s* |  | 4.00 *s* | 3.95 *s* | 3.91 *s* |
| $OCOC\underline{H}_3$ | — | — | 2.12 *s* | 2.07 *s* | 2.08 *s* | 2.10 *s* | — | — | 2.10 *s* |
| $OCOCH_2CH(C\underline{H}_3)_2$ | 0.94 *d* | 0.96 *d* | — | — | — | — | — | — | — |
|  | (6.2) | (6.2) |  |  |  |  |  |  |  |
| $OCOC\underline{H}_2C\underline{H}(CH_3)_2$ | 2.00–2.30 *m* | 2.00–2.40 *m* | — | — | — | — | — | — | — |
| OH | — | 11.13 *s* | 11.09 *s* | — | — | — | 11.19 *s* | — | — |
| $OCH_3$ | 3.83 *s* | — | — | 3.82 *s* | — | — | — | 3.87 *s* | 3.87 *s* |

Table 4. $^{13}C$ NMR (50 MHz) ($CDCl_3$) of compounds **1–8**

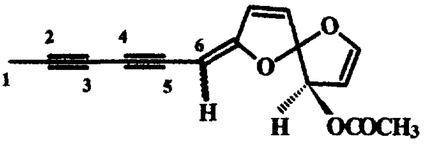
| Carbon | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
|---|---|---|---|---|---|---|---|---|
| 1 | 62.89 *d* | 63.67 *d* | 64.50 *d* | — | 63.59 *d* | 65.80 *d* | 26.71 *t* | 175.71 *s* |
| 2 | 72.27 *s* | 72.00 *s* | 72.08 *s* | 78.01 *s* | 72.36 *s* | 72.13 *s* | 73.64 *s* | 70.02 *s* |
| 3 | 70.29 *s* | 71.15 *s* | 71.41 *s* | 72.84 *s* | 71.53 *s* | 70.93 *s* | 67.54 *s* | 87.15 *s* |
| 4 | 78.44 *s* | 78.25 *s* | 78.85 *s* | 79.38 *s* | 78.73 *s* | 78.43 *s* | 74.33 *s* | 79.02 *s* |
| 5 | 63.55 *s* | 63.57 *s* | 68.67 *s* | 70.44 *s* | 64.04 *s* | 63.52 *s* | 64.29 *s* | 63.31 *s* |
| 6 | 4.26 *q* | 4.30 *q* | 4.67 *q* | 4.22 *q* | 4.66 *q* | 4.23 *q* | 4.21 *q* | 4.89 *q* |
| 1′ | 135.00 *s* | 138.01 *s* | 138.25 *s* | 135.19 *s* | 137.68 *s* | 136.38 *s* | 138.05 *s* | 134.87 *s* |
| 2′ | 122.10 *s* | 110.61 *s* | 111.08 *s* | 122.62 *s* | 128.93 *s* | 127.69 *d* | 111.36 *s* | 123.32 *s* |
| 3′ | 156.70 *s* | 162.48 *s* | 162.92 *s* | 157.03 *s* | 131.15 *d* | 128.62 *d* | 162.88 *s* | 156.61 *s* |
| 4′ | 111.92 *d* | 118.95 *s* | 119.52 *d* | 112.43 *d* | 129.18 *d* | 129.02 *d* | 117.17 *d* | 116.93 *d* |
| 5′ | 130.99 *d* | 134.56 *d* | 135.01 *d* | 131.41 *d* | 132.91 *d* | 128.62 *d* | 134.57 *d* | 130.50 *d* |
| 6′ | 120.24 *d* | 119.83 *s* | 120.32 *d* | 120.17 *d* | 128.97 *d* | 127.69 *d* | 121.53 *d* | 124.13 *d* |
| $\underline{C}OOCH_3$ | 166.88 *s* | 170.52 *s* | 169.76 *s* | 167.18 *s* | 167.31 *s* | — | 171.25 *s* | 167.60 *s* |
| $COO\underline{C}H_3$ | 52.45 *q* | 52.80 *q* | 53.19 *q* | 52.58 *q* | 52.75 *q* | — | 52.43 *q* | 52.82 *q* |
| $O\underline{C}OCH_2CH(CH_3)_2$ | 171.22 *s* | 171.42 *s* | — | — | — | — | — | — |
| $OCO\underline{C}H_2CH(CH_3)_2$ | 42.90 *t* | 43.14 *t* | — | — | — | — | — | — |
| $OCOCH_2\underline{C}H(CH_3)_2$ | 25.58 *d* | 25.72 *d* | — | — | — | — | — | — |
| $OCOCH_2CH(\underline{C}H_3)_2$ | 22.29 *q* | 22.36 *q* | — | — | — | — | — | — |
| $OCH_3$ | 56.10 *q* | — | — | 56.28 *q* | — | — | — | 56.38 *q* |
| $OCO\underline{C}H_3$ | — | — | 21.30 *q* | 20.68 *q* | 21.18 *q* | 20.90 *q* | — | — |
| $O\underline{C}OCH_3$ | — | — | 170.88 *s* | 169.32 *s* | 169.62 *s* | 169.55 *s* | — | — |

*Extraction and isolation*. Root bark (0.75 kg) of *A. frutescens* (wild specimens) collected in Rio de Arico, was extracted with cold $Me_2CO$ (2 l). The extract (30.8 g) was repeatedly chromatographed on Sephadex LH-20 and silica gel using mixts, of petrol–$CHCl_3$–MeOH (2:1:1) and petrol–EtOAc, respectively, to afford the following products: **1** (5 mg), **2** (9 mg), **3** (112 mg), **4** (58 mg), **5** (15 mg), **6** (6 mg), **7** (7 mg), **8** (13.8 mg), **9** (4 mg), **10** (12 mg) and **11** (3 mg).

*Frutescinol isovalerate* (**1**). Yellow oil. $[\alpha]_D^{20}$: +0.14 (*c* 1.1, $CHCl_3$). UV $\lambda_{max}^{EtOH}$ nm: 304, 251, 242, 238. IR $\nu_{max}$ (film) $cm^{-1}$: 2900, 1730, 1580, 1470, 1430, 1260,



**1** $R_1$=$OCOCH_2CH(Me)_2$; $R_2$=$COOCH_3$; $R_3$=OMe; $R_4$=Me
**2** $R_1$=$OCOCH_2CH(Me)_2$; $R_2$=$COOCH_3$; $R_3$=OH; $R_4$=Me
**3** $R_1$=OAc; $R_2$=$COOCH_3$; $R_3$=OH; $R_4$=Me
**4** $R_1$=OAc; $R_2$=$COOCH_3$; $R_3$=OMe; $R_4$=Me
**5** $R_1$=OAc; $R_2$=$COOCH_3$; $R_3$=H; $R_4$=Me
**6** $R_1$=OAc; $R_2$=H; $R_3$=H; $R_4$=Me
**7** $R_1$=H; $R_2$=$COOCH_3$; $R_3$=OMe; $R_4$=Me
**8** $R_1$=(=O); $R_2$=$COOCH_3$; $R_3$=OMe; $R_4$=Me
**9** $R_1$=OAc; $R_2$=$COOCH_3$; $R_3$=OMe; $R_4$=H



**10** 6E
**11** 6Z

Table 5. Minimal inhibitory concentration of compounds **3**–**5** from *A. frutescens*

| Compounds | Microorganisms (MIC $\mu$g ml$^{-1}$) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S.a. | S.s. | S.e. | S.al. | M.l. | B.s. | K.p. | P.m. | S.t. | E.c. |
| **3** | >50 | 15 | 3 | 6 | 2 | 6 | 20 | 25–50 | 35 | 20 |
| **4** | >50 | >50 | >50 | >50 | 15 | >50 | >50 | >50 | >50 | >50 |
| **5** | 40 | 30–20 | 15 | >50 | 8–10 | >50 | >50 | >50 | >50 | >50 |

Compounds **2**, **6**–**8** and **10** were inactive at <50 $\mu$g ml$^{-1}$ against Gram-positive and Gram-negative bacteria assayed.

All compounds inactive at <50 $\mu$g ml$^{-1}$ against the yeasts, *C. albicans* and *S. cerevisiae*.

Table 6. Cytotoxic activity of compounds **2**–**5** and **10** from *A. frutescens*

| Compounds | $IC_{50}$ ($\mu$g ml$^{-1}$) | |
|---|---|---|
| | HeLa | Hep-2 |
| **2** | 37 | >50 |
| **3** | 30 | 29 |
| **4** | 48 | >50 |
| **5** | 18 | 26 |
| **10** | 44 | >50 |
| Control | 0.1 | 0.6 |

Compounds **6**–**8** inactive at <50 $\mu$g ml$^{-1}$.
Mercaptopurine used as positive control.
Results are mean values from three determinations.

1150, 1110, 1070 cm$^{-1}$. EI-MS *m/z* (rel. int.): 342 $[M]^+$, (4), 257 (100), 226 (19); HR-EIMS *m/z* 342.146394 (cal. for $C_{20}H_{22}O_5$, 342.146724).

*3'-Demethylfrutescinol isovalerate* (**2**). Yellow oil. $[\alpha]_D^{20}$: +38.6 (*c* 1.4, $CHCl_3$). UV $\lambda_{max}^{EtOH}$ nm: 304, 251, 242, 238. IR $\nu_{max}$ (film) cm$^{-1}$: 2959, 1735, 1747, 1671, 1590, 1450, 1265. EI-MS *m/z* (rel. int.): 328 ($[M]^+$, 6), 243 (100), 212 (81), 196 (12); HR-EIMS *m/z* 328.131104 (cal. for $C_{19}H_{20}O_5$, 328.131074).

*Antimicrobial assay*. Fourteen organisms were used for antimicrobial activity: *Staphylococcus aureus* ATCC 6538, *S. epidermidis* CECT 232, *S. albus* SUBC 1, *Bacillus subtilis* CECT 39, *B. pumilus* CECT 29, *B. megaterium* CECT 44, *Micrococcus luteus* CECT 241, *Escherichia coli* CECT 99, *Salmonella typhimurium* STBC 1, *Pseudomonas aeruginosa* AK 958, *Proteus mirabilis* CECT 170, *Klebsiella pneumoniae* CECT 367, *Candida albicans* UBC 54 and *Saccharomyces cerevisiae* X2180A. The strains were maintained on Nutrient Agar (Oxoid) or Sabouraud Dextrose Agar (Oxoid). Bacterial cultures were developed in Nutrient Broth (Oxoid) and the yeast culture in YEPD medium, composition l$^{-1}$ as follows: yeast extract (Oxoid) 10 g, peptone (Oxoid) 10 g and glucose 20 g. Inocula were prepd by diluting cultures 1:100 or 1:1000 with sterile distilled $H_2O$ overnight. Large plates (23 × 23 cm) were prepd with Nutrient Broth (Oxoid) for bacteria and Sabouraud medium for yeast, and inoculated with 1 ml of diluted culture. Excess inoculum was removed and the plates dried for 30 min at 37°. Holes (6 mm in diameter) were made in the inoculated agar and filled with 20 $\mu$l of crude extracts (10 mg ml$^{-1}$) dissolved in DMSO; ampicillin and streptomycin (10 $\mu$g hole$^{-1}$) were used as positive controls. One hole was filled with 20 $\mu$l of DMSO and used as a negative control. The diameter of the zones of inhibition in mm were recorded after 24 hr of incubation at 37° or, in the case of the yeast, at 28°. Each expt was performed in triplicate. Minimal inhibitory concns (MIC) of the different products isolated from *A. frutescens* were determined in liquid medium as previously described [7]. Products were added in a soln of DMSO and tubes containing the same proportions of DMSO were used as controls.

*Cytotoxic assay.* HeLa (human carcinoma of cervix) and Hep-2 (human carcinoma of larynx) cell lines were grown as a monolayer in Dulbecco's modified Eagle's medium, DMEM (Gibco), supplemented with 10% newborn calf serum (Gibco) and 1% of penicillin-streptomycin mixt. (10 000 UI ml$^{-1}$). Cells were maintained at 37° in 5% $CO_2$ and 80% humidity. Cytotoxicity of crude extracts and compounds isolated from *A. frutescens*, was assessed using the colorimetric MTT reduction assay [8]. Two-fold dilns of plant extracts or pure compounds were prepd in 96-microwell plates and added to each well; culture medium with 1% DMSO (the highest concn used in the test) was added to control wells and culture medium alone without cells were used as blanks. Mixts were incubated for 24 hr at 37° in a 5% $CO_2$-incubator before adding 10 $\mu$l of a stock soln of MTT to each well and continuing incubation for a further 2 hr. Acid-*iso*ProH (150 $\mu$l) was added to solubilize the MTT formazan and the A of each well was measured using a micro ELISA reader at 600 nm. Percentage of viability was plotted against the different concns and the 50% cell viability ($IC_{50}$) was calcd from the curve. Expts were repeated at least three times.

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