Synthesis and Bioactivity of Novel Acetylenic Compounds

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The synthesis of novel acetylenic ketone compounds and anti-inflammatory and antimicrobial activities are herein described.

It is well known that acetylenic compounds have remarkable antimicrobial activity.¹⁾ However, to our surprise, we have also found that acetylenic compounds, particularly conjugated acetylenic ketones, have marked anti-inflammatory activity.

In the course of our intensive research to discover new anti-inflammatory agents in natural products, we isolated a very active substance, which was proved to be Caplillin reported previously by Imai.²⁾ We then discovered that some of the known synthetic acetylenic compounds³⁾ also have anti-inflammatory activity.

Based on this new knowledge, we synthesized a large number of the various classes of novel acetylenic compounds and examined their anti-inflammatory activity, gastrointestinal disturbance and antimicrobial activity.

MATERIALS AND METHODS

1) *Synthesis of monoacetylenic ketones.* The general method I, involving the alkylation of aldehydes with ethynyl magnesium bromides⁴⁾ and oxidation of propargylic alcohols to conjugated ketones was used as described in the following scheme

CHART 1.

CHART 2.

In a few cases, a direct ketone synthesis method was used as described below;

A representative example is the following;
4-Phenoxy-tetrolophenone

A solution of ethyl magnesium bromide which was prepared from 6 g of ethyl bromide and 1.3 g of magnesium in 50 ml of anhydrous tetrahydrofuran was added to a solution of 6.8 g of 1-phenoxy-2-propyne in 10 ml of anhydrous tetrahydrofuran at room temperature. After stirring for 2 hr, the reaction mixture was cooled to below 0°C and 5.3 g of benzaldehyde was then added under cooling and the mixture was left overnight.

Decomposition of the complex with 50 ml of saturated aqueous ammonium hydrochloride, dilution with water, acidification with hydrochloric acid, extraction with ether, washing with water, drying and evaporation of the solvent yielded 12.0 g of crude 1-phenyl-1-hydroxy-4-phenoxy-2-butyne as a solid, which was dissolved in 300 ml of anhydrous acetone. To this solution was added 120 g of active manganese dioxide and the mixture was vigorously stirred for 4 hr at room temperature under an atmosphere of nitrogen. Removal of manganese dioxide with a Celite column, evaporation of the solvent, crystallization with a mixture of ether and *n*-hexane and recrystallization from a mixture of acetone and methanol yielded 7.8 g of 4-phenoxy-tetrolophenone. mp 59.5~61.0°C IR $\nu_{\text{max}}^{\text{Nujol}}$: 2230 cm⁻¹, 1650 cm⁻¹. NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ 4.92 (s, 2H), aromatic protons. Anal. Found: C, 81.59;

H, 4.94. Calcd. for $C_{16}H_{12}O_2$: C, 81.34; H, 5.12.

2) *Synthesis of diacetylenic ketones.* For the preparation of diacetylenic ketones, the general method II was used. This involves Cadiot-Chodkiewicz coupling⁵⁾ of propargylic alcohols and 1-bromoacetylenic compounds followed by oxidation, as shown below;

CHART 3.

A representative example of the synthetic method is described as follows;

5-(1-Cyclohexenyl)-2,4-pentadiynophenone.

A solution of 13 g of phenylethynylcabinol, 50 ml of *n*-butyl-amine, 200 mg of cuprous chloride, 12 g of hydroxylamine hydrochloride in 200 ml of 99% ethanol was prepared. To this a solution of 18.5 g of 1-cyclohexenyl bromoacetylene in 100 ml of ethanol at 18~20°C was added dropwise under cooling. After stirring for 30 min, 1 g of sodium cyanide was added and the reaction mixture was diluted with water. Extraction of the mixture with ether, washing successively with aqueous sodium carbonate, hydrochloric acid and water, drying and evaporation of the solvent yielded 9.8 g of crude 1-hydroxyl-5(1-cyclohexenyl)-2,4-pentadiyne.

$$n_D^{24}=1.6144 \text{ IR } \nu_{\text{max}}^{11q}: 3350 \text{ cm}^{-1}, 2250 \text{ cm}^{-1}$$

This oil was dissolved in 200 ml of dried acetone and stirred vigorously with 100 g of active manganese dioxide for 4 hr at room temperature under an atmosphere of nitrogen. Removal of manganese dioxide from the reaction mixture by filtration and evaporation of the solvent, gave a brown oil, which was chromatographed on silicic acid to yield 4.1 g of 5-(1-cyclohexenyl)-2,4-pentadiynophenone, as a pale brown oil. $n_D^{26}=1.6590$. Anal. Found: C, 86.88; H, 6.09. Calcd. for $C_{17}H_{14}O$: C, 87.15; H, 6.02. IR ν_{max}^{11q} : 2215 cm^{-1} , 2145 cm^{-1} , 1640 cm^{-1} . NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 1.62 (m, 4H), 2.17 (m, 4H), 6.47 (q, 1H), 7.3~7.7 (m, 3H) 8.15 (m, 2H).

3) *Anti-inflammatory activity.* Animals used in these tests were fed the pellet diet (CE-2) supplied by CLEA and drinking water *ad libitum*.

a) *Carrageenin-induced edema.* Three male rats of the Wistar strain weighing 150~170 g were used for each dose level. According to Winter's method,⁶⁾ 0.05 ml of a 1% carrageenin suspension in a sterile saline solution was injected into the plantar tissue of the right hind paw. Test materials suspended in 0.5% carboxymethylcellulose (CMC) were administered oral-

ly 1 hr before the carrageenin injection and the foot volume was determined with a plethysmometer (Plethysmograph $\Delta V-3$, Ugo Basile, Milano) 4 hr after injection of Carrageenin.

b) *Gastric toxicity.* The rats which were used in the carrageenin edema test were dissected 72 hr after determination of the foot volume and the effect of the test compound on the gastro-intestinal tract was observed visually.

Degree of toxicity was determined as follows;

- (+++): Serious ulcer, bleeding; body-weight loss
- (++) : Medium ulcer, bleeding; body-weight loss
- (+) : Slight bleeding, congestion: slight body-weight loss
- (±) : Very slight congestion or no other change; no weight loss

4) *Antimicrobial activity.* The tests were performed by the agar streak dilution technique using two fungi and one bacteria as test organisms. Each compound, dissolved in acetone in adequate concentration, was diluted with the culture medium to the final concentration. In all cases the final concentration of acetone was less than 0.5%.

The antimicrobial activity of each test compound was expressed in terms of the minimal inhibitory concentration (MIC) required for complete inhibition of the test microorganism after the incubation period.

The culture conditions for the microorganisms are shown below;

Strain	Medium	Temperature (°C)	Incubation period, (day)
<i>Candida albicans</i>	Sabouraud agar	30	3
<i>Trichophyton rubrum</i>	"	"	7
<i>Staphylococcus</i> 209 P	Nutrient agar	37	1

RESULT AND DISCUSSION

1) *Anti-inflammatory activity*

1. Generally speaking, non-steroidal anti-inflammatory agents often have such side effects on the gastro-intestinal system as gastric hemorrhage and intestinal perforation.⁷⁾ In the case of acetylenic ketones, too, as revealed in Table I, all the compounds show several grades of gastro-intestinal injury at effective dose (over 30% inhibition). However, the degrees of gastric injury were so different from each other that we obtained some examples in

TABLE I. INHIBITION OF CARAGEENIN-INDUCED EDEMA IN RATS AND GASTRIC TOXICITY CAUSED BY ACETYLENIC KETONES

No.	Compound	mp n_D	Inhibition (%)					Toxicity	
			10	20	50	100	200	400	mg/kg Degree
1	 Capillin	2)	18	46	61	78	90	20	+
	Phenylbutazone				30	41	63	50	+
2		1)				19	41	48	100 ±
3		3)	17	27	41		60	200	±
4		79.5°C					7	17	400 —
5		99°C				19	41	48	100 ±
6		65°C					0	14	400 —
7		109.5°C			15		32	200	—
8		133°C						24	400 —
9		80°C					12	17	400 —
10		60°C						38	400 —
11		102°C					21	20	400 —
12		n_D^{26} : 1.6590			23	51	73	100	±

No.	Compound	Dose mg/kg mp n_D	Inhibition (%)						Toxicity	
			10	20	50	100	200	400	mg/kg	Degree
13		72.5°C					45		200	±
14		n_D^{27} : 1.6550					39	44	200	±
15		n_D^{24} : 1.6009					35	41	200	±
16		n_D^{26} : 1.5979			43		49	49	50	±
17		3)	11	45	69		91		20	+
18		71°C		8	31		88		50	+
19		86°C			45		82		50	++
20		72°C			0	16	62	82	50	++
21		36.5°C			63		90		50	++
22		65°C					0	21	400	—
23		72°C						41	400	—
24		n_D^{21} : 1.5936					0	0	400	—
25		n_D^{26} : 1.5175					57		200	++
26		1)	0	46	83		98	98	20	+

No.	Compound	mp n_D	Inhibition (%)						Toxicity	
			10	20	50	100	200	400	mg/kg	Degree
27		n_D^{23} : 1.5196			0		15	47	50	±
28					16	27	29	40	50	±
29		61°C					6	49	400	+
30		45°C					36	31	400	±
31		55°C					0	0	400	—
32		41°C					22	30	200	±
33		62°C						19	400	—
34		55°C						15	400	—
35		60°C						9	400	—
36		100°C						11	400	—
37		n_D : 1.5395					80		200	+++
38		n_D^{27} : 1.6050			11		28		200	±
39		n_D^{23} : 1.5670					62	69	200	+++
40		n_D^{26} : 1.5841	11	22	85				50	+++

No.	Compound	Dose mg/kg mp n_D	Inhibition (%)					Toxicity	
			10	20	50	100	200	400	mg/kg Degree
41		n_D^{25} : 1.6150	12	55	90		90	10	+
42		n_D^{25} : 1.6170		33	45		92	200	+++
43		n_D^{25} : 1.5069		38			90	20	+
44		n_D^{25} : 1.5702					29	40	400 -

which separation of anti-inflammatory activity from gastric toxicity were reasonably successful. Cycloalkenyl diynophenones (Compound Nos. 12, 13, 14), No. 30 and No. 42 showed neither body-weight loss nor gastric hemorrhage at effective dose.

2. Generally speaking the monoacetylenic ketones may be considered more toxic than the corresponding diacetylenic ketones when Compound Nos. 1, 3, 10, 12 and 16 are compared with Nos. 26, 28, 29, 38 and 39, respectively.

3. Any substituent on a phenyl ring, whether electron-donating or electron-withdrawing, markedly reduced both anti-inflammatory activity and toxicity in any class of compounds, such as the phenyldiynophenone group (Compound Nos. 3 to 8), the phenoxydiynophenone group (Compound Nos. 8 to 9), or the phenoxytetrolphenone group (Compound Nos. 29 to 36).

4. Introduction of vinylogous phenyl (Nos. 9, 41), alkyl (Nos. 25, 37, 44) or cycloalkyl (Nos. 24, 43) instead of phenyl in the phenone structure, causes no improvement on either activity or toxicity.

5. There may be a possibility that further elongation of the conjugated ynone system will reduce toxicity without much decrease of anti-inflammatory activity, as inferred from results, 1 and 2. But the stability of the compound

will be much reduced, too.

6. In conclusion, we can infer that there is a close relation between the anti-inflammatory activity of acetylenic ketones and the irritating action on gastro-intestinal tract, and both the activity and the adverse effect from the ynone system, and that the toxicity can be reduced by substitution of the system or elongation of its conjugation.

2) Antimicrobial activity

1. As shown in Table II, the novel acetylenic ketones which we obtained in this work reveal higher activity against *Trichophyton rubrum* than against *Candida albicans* or *Staphylococcus aureus* in general agreement with the data, reported by Tanaka *et al.*¹⁾

Oxygen substituted ynophenones, for example, Compound Nos. 17, 21, 15, 34 and 35 were relatively effective against *Candida albicans* and Compound No. 15, a representative compound, showed some therapeutic effect on rats by oral administration in an *in vivo* infection experiment.

2. We cannot perceive any relation between anti-*Trichophyton* activity and gastric toxicity, which may be considered to be local irritating action. Therefore, it can be said that we have obtained a number of novel compounds which are substantially as active as Capillin and much

TABLE II. ANTIMICROBIAL ACTIVITIES OF ACETYLENIC KETONES

No.	Test organisms Compound	MIC (mcg/ml)		
		<i>Candida albicans</i>	<i>Trichophyton rubrum</i>	<i>Staphylococcus aureus</i> 209p
	Nystatin	1.56 ~ 6.25		
	Griseofulvin		1.56 ~ 6.25	
	Capillin	3.13	0.1	
27		> 200	6.25	
3		> 400	1.56	
4		100	3.13	
6		> 400	6.25	
7		400	25.0	
8		> 400	25.0 ~ 50	
10		25	0.78 ~ 3.13	
12		400	3.13 ~ 6.25	
13		> 100	6.25	
15		6.25		
16		> 100	0.39 ~ 1.56	
17		3.13	0.2	
18		25 ~ 100	1.56 ~ 6.25	

No.	Test organisms		MIC (mcg/ml)		
	Compound	<i>Candida albicans</i>	<i>Trichophyton rubrum</i>	<i>Staphylococcus aureus</i> 209p	
19		6.25	1.56		
20		6.25 ~ 25	0.39 ~ 1.56		
21		3.13 ~ 1.56	1.56		
22		25 ~ 100	1.56 ~ 6.25		
11		> 400	1.56 ~ 6.25		
29		25	6.25		
30		50	50		
32		12.5		25	
33		12.5		12.5	
34		6.25		25	
35		6.25		25	
36		400	400		
37		6.25	3.13		
25		> 400	> 400		

No.	Compound	Test organisms			MIC (mcg/ml)		
					<i>Candida albicans</i>	<i>Trichophyton rubrum</i>	<i>Staphylococcus aureus</i> 209p
23		50			0.78 ~ 3.13		
41		50			6.25		
42		50			3.13		
38		25			6.25		
39		12.5			1.56		
45		> 100			25 ~ 100		
43		50			3.13		

less irritative than Capillin, for example, Compound Nos. 4, 10, 11, 12, 16, 22, 23 and 42.

3. We cannot apply structure-activity relationship in any other effect than anti-inflammatory activity. Therefore, antimicrobial activity and anti-inflammatory activity of acetylenic ketones are intrinsically different.

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REFERENCES

- 1) J. Rrisch, W. Spitzner and K. E. Schulte, *Arznei-mittel Forschung*, **17**, 816; K. Tanaka, I. Iwai, Y. Okajima and T. Konotsune, *Antibiotics and Chemotherapy*, **9**, 151 (1959).
- 2) K. Imai, *YAKUGAKU ZASSHI*, **76**, 405 (1956).
- 3) B. W. Nash and T. D. Williams, *J. Chem. Soc.*, **1965**, 2983.
- 4) E. R. H. Jones, L. Skatteböl and M. C. Whiting, *J. Chem. Soc.*, **1956**, 4765.
- 5) G. Eglinton and W. Mearce, "Advances in Organic Chemistry," vol. 4, John Wiley & Sons, 1963, p. 225.
- 6) C. A. Winter, E. A. Risley and G. W. Guss, *Proc. Soc. Exptl. Biol. Med.*, **111**, 544 (1962).
- 7) H. Mizushima, "Inflammation and Anti-inflammatory Agent," Nankodo, Japan, 1970.

1) J. Rrisch, W. Spitzner and K. E. Schulte, *Arznei-*