

## Biological Activity of Cannabichromene, its Homologs and Isomers

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**Abstract:** Cannabichromene (CBC) is one of four major cannabinoids in *Cannabis sativa* L. and is the second most abundant cannabinoid in drug-type cannabis. Cannabichromene and some of its homologs, analogs, and isomers were evaluated for antiinflammatory, antibacterial, and antifungal activity. Antiinflammatory activity was evaluated by the carrageenan-induced rat paw edema and the erythrocyte membrane stabilization method. In both tests, CBC was superior to phenylbutazone. Antibacterial activity of CBC and its isomers and homologs was evaluated using gram-positive, gram-negative, and acid-fast bacteria. Antifungal activity was evaluated using yeast-like and filamentous fungi and a dermatophyte. Antibacterial activity was strong, and the antifungal activity was mild to moderate.

CRUDE drugs from the cannabis plant have been used by man for over 5000 years. Some of these crude drugs have been associated with varying degrees of medical applications in many countries. However, as new and better medicinal agents were discovered, these crude drugs lost favor in formal medical clinics. Interest in these crude drugs has recently been renewed. The U.S. Federal Government, since 1968, has sponsored considerable research on cannabis (mostly marihuana and hashish) and synthetic cannabinoids, notably (-)-*trans*- $\Delta^9$ -tetrahydrocannabinol (THC), (-)-*trans*- $\Delta^8$ -tetrahydrocannabinol ( $\Delta^8$ -THC), cannabinol (CBN), and cannabidiol (CBD). Due to the problems associated with quantitating the quality of any crude drug, particularly marihuana in the smoke form, much of the early laboratory work was done with synthetic cannabinoids. Early researchers, using pure synthetic cannabinoids, found leads to support some of the anecdotal reports about the crude drug marihuana. Although these laboratory findings were primarily for the four previously mentioned synthetic cannabinoids, very little work

was carried out on mixtures of these compounds in the ratios in which they occur in the crude drugs. Also, the "most active" ingredient, THC, was considered to be the only significant compound in understanding the pharmacologic action of marihuana. Actually, marihuana contains many compounds that are active in biologic systems.<sup>1</sup>

Cannabis is a complex plant which contains 421 known compounds from 18 different chemical classes. Sixty-one cannabinoids and their homologs, as defined by Mechoulam,<sup>2</sup> are known to occur in the plant.<sup>3</sup> These 61 cannabinoids can be further classified into at least 15 different types. Ten of these cannabinoids can be quantitated routinely using methods reported in the literature.<sup>4</sup> The chemical composition of crude drugs from cannabis is much better understood today, and it is obvious that the THC content of marihuana cannot be used to adequately describe the pharmacologic activity of the drug. In an attempt to integrate analytic and biologic data on the major, naturally occurring cannabinoids, it was discovered that cannabidiol was totally absent in many cannabis samples and present in various amounts in others.<sup>5</sup> Cannabichromene was

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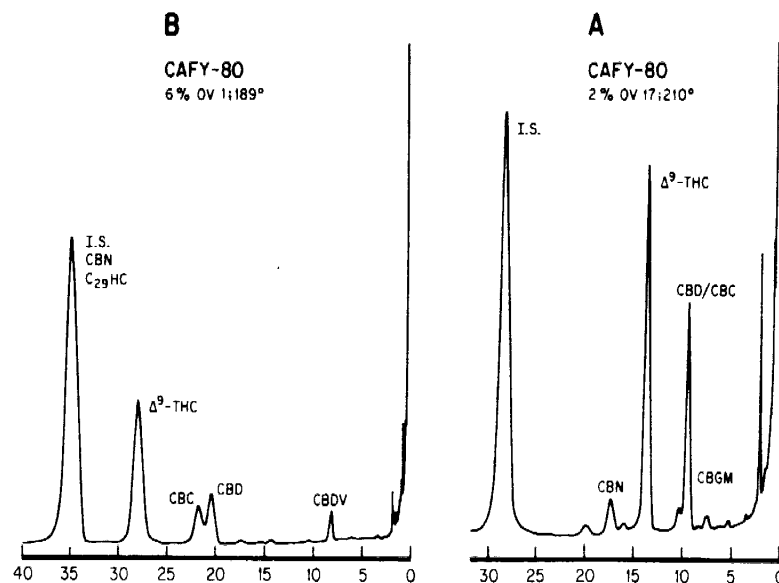


Fig. 1. Gas-chromatographic analysis of the cannabinoids in a chloroform extract of Afghanistan cannabis using 2% OV-17 (A) and 6% OV-1 (B) columns. For details regarding the chromatographic conditions, see Refs. 4 and 5.

identified as the cannabinoid under the chromatogram peak labeled cannabidiol in drug-type cannabis, for example, Mexican, Costa Rican, Brazilian, Jamaican, and South African. Cannabidiol and cannabichromene were present in nearly equal

amounts in certain types; for example, Afghanistani and Pakistani. In other samples, cannabidiol was the major cannabinoid under the peak, with a trace of cannabichromene; for example, Iranian, Lebanese, and Turkish. See Fig. 1 for gas-

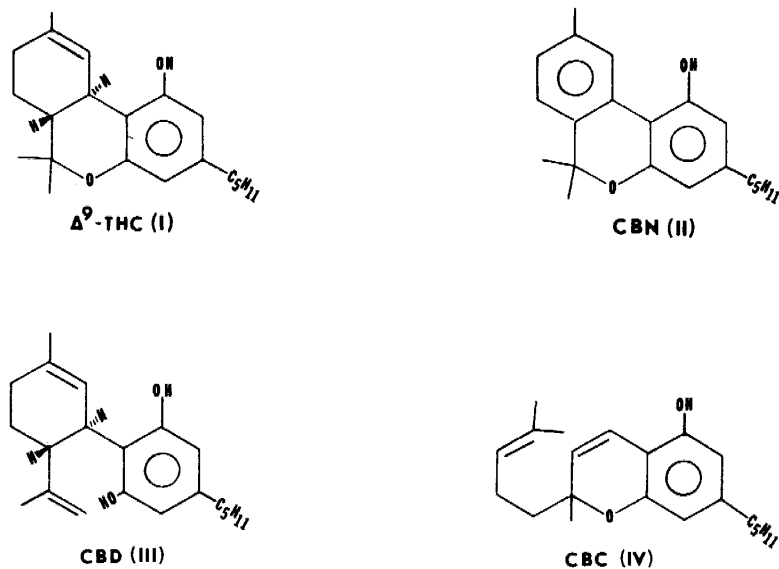


Fig. 2. Structures of the major cannabinoids found in *Cannabis sativa* L.

chromatographic separation of cannabidiol and cannabichromene on two different columns using Afghanistan cannabis extracts.

Cannabichromene (CBC), along with THC, cannabidiol, and cannabinol (Fig. 2), are thus the most abundant naturally occurring cannabinoids. In freshly harvested drug-type cannabis material, THC and cannabichromene are the major cannabinoids. Cannabinol is a product of the decomposition of THC.<sup>6</sup>

In view of these findings regarding the abundance of cannabichromene in drug-type cannabis and after developing a synthetic method to produce large quantities of this naturally occurring cannabinoid,<sup>7</sup> an extensive biologic study on cannabichromene was initiated in our laboratories. In this connection, we have reported data indicating that cannabichromene, in monkeys, potentiates the mind-altering properties of THC.<sup>8</sup> In this report, data on cannabichromene and its homologs and isomers are confined to the following three areas: (1) antiinflammatory, (2) antibacterial, and (3) antifungal.

## Methods

### *Preparation of Cannabichromene (IV) and Its Homologs and Isomers (V-VIII)*

Cannabichromene (IV), cannabichromene-C<sub>1</sub> (VI), and cannabichromene-C<sub>0</sub> (VII) were prepared by condensation of citral with olivetol, orcinol, and resorcinol, respectively, following our previously published method for synthesis of cannabichromene.<sup>7</sup> The yield for VI was 48.3 per cent while that of VII was only 10.8 per cent.

Isocannabichromene-C<sub>0</sub> (VIII) was separated from the same reaction mixture of VII as a side product. However isocannabichromene (V) was prepared by condensing olivetol and citral in pyridine at 110° for 7 hours,<sup>9</sup> where both IV and V were formed along with other side products.

All compounds were purified by column chromatography over silica gel and were characterized by spectral methods including MS, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, IR and UV.

### *Determination of Acute LD<sub>50</sub> of Cannabichromene*

ICR mice (Laboratory Supply Company, Inc., Indianapolis, Ind.) weighing 30 to 35 Gm were used to determine the LD<sub>50</sub> of cannabichromene using two routes of administration—subcutaneous and oral. Drug-dosed mice were observed for seven days. Cannabichromene was emulsified with gum acacia (5%) and olive oil (10%) in water. A vehicle made in the same manner showed no lethality over a two-week period.

### *Antiinflammatory Screening*

Two methods were used to assess the antiinflammatory activity of cannabichromene: the carrageenan-induced rat paw edema<sup>10</sup> and erythrocyte membrane stabilization assay.<sup>11</sup> Groups of eight Sprague-Dawley rats (Laboratory Supply Company, Inc., Indianapolis, Ind.) were used for the carrageenan-induced rat paw edema assay. Rats of both sexes were used in performing initial assays, and results for both sexes were identical. Thus, male rats were used for the remainder of the study. Intraperitoneal and oral routes were used. All animals received a 750 mg/kg intraperitoneal injection of ethylurethane 1 hour before testing to render them tractable. Negative control groups received the vehicle, while positive control groups received a dose of phenylbutazone (PBZ) as shown in Table II. The test compounds were prepared for dosing by emulsification in 3% arlacel and 2% Tween-60 in water.

The published procedure was followed for the erythrocyte membrane stabilization method.<sup>11</sup> All compounds were prepared by dissolving in physiologic saline or as a fine suspension in 2% ethanol in saline and the pH adjusted to 7.4. The concentrations

TABLE I  
Antimicrobial Screening Using the  
Agar Well Diffusion Assay

Organism	ATCC#	Classification
<i>Bacillus subtilis</i>	6633	gram-positive bacterium
<i>Staphylococcus aureus</i>	6538	gram-positive bacterium
<i>Escherichia coli</i>	10536	gram-negative bacterium
<i>Pseudomonas aeruginosa</i>	15442	gram-negative bacterium
<i>Mycobacterium smegmatis</i>	607	acid-fast bacterium
<i>Candida albicans</i>	10231	yeast-like fungus
<i>Saccharomyces cerevisiae</i>	9763	yeast-like fungus
<i>Aspergillus niger</i>	16888	filamentous fungus
<i>Trichophyton mentagrophytes</i>	9972	dermatophyte

tested are given in Table III. Apirin (ASA) and phenylbutazone were used as positive controls for comparison of activity.

*Antimicrobial Screening  
(antibacterial and antifungal)*

All compounds were screened for activity against the organisms listed in Table I.

Both qualitative and quantitative screens were performed. Qualitative screening was accomplished using the agar well diffusion assay, and quantitative screening was accomplished by the two-fold (broth) serial dilution method to determine the minimum inhibitory concentration (MIC). Inoculation was accomplished with one loop-full of a 1:10 dilution of the 24-hour-old broth cul-

TABLE II  
Inhibition of Carrageenan-Induced Rat Paw Edema by  
Cannabichromene and its C<sub>1</sub>-Homolog

Compound	Dose (mg/kg)	Route of administration	Inhibition (%)
Cannabichromene (IV)	120	intraperitoneal injection	70*
	240	intraperitoneal injection	86*
	480	intraperitoneal injection	100
Cannabichromene-C <sub>1</sub> (V)	60	intraperitoneal injection	53*
	120	intraperitoneal injection	63*
	240	intraperitoneal injection	92*
Phenylbutazone	60	intraperitoneal injection	52*
	120	intraperitoneal injection	76
Cannabichromene (IV)	120	oral, fasted rats	35 (N.S.)
	240	oral, fasted rats	67*
	480	oral, fasted rats	55**
Phenylbutazone	120	oral, fasted rats	36 (N.S.)
Cannabichromene (IV)	120	oral, nonfasted rats	21 (N.S.)
	240	oral, nonfasted rats	34**
	480	oral, nonfasted rats	50*
Phenylbutazone	60	oral, nonfasted rats	37*
	120	oral, nonfasted rats	22*

\*  $P = 0.01$ .

\*\*  $P = 0.05$ .

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TABLE III

Inhibition of Heat-Induced Erythrocyte Hemolysis  
by Cannabichromene and its Homologs and Isomers

Compound	Concentration of test solution (M)	Inhibition (%)
Cannabichromene (IV)	$1 \times 10^{-4}$	98
	$5 \times 10^{-5}$	79
	$2.5 \times 10^{-5}$	67
	$1.25 \times 10^{-5}$	55
Isocannabichromene (VII)	$1 \times 10^{-4}$	75
Cannabichromene- $C_1$ (V)	$1 \times 10^{-4}$	86
Cannabichromene- $C_0$ (VI)	$1 \times 10^{-4}$	69
Isocannabichromene- $C_0$ (VIII)	$1 \times 10^{-4}$	43
Phenylbutazone	$1 \times 10^{-4}$	16
	$2 \times 10^{-5}$	10
Aspirin	$2.5 \times 10^{-4}$	21

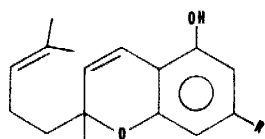
ture\* of the test organisms in sterile water. Streptomycin sulfate was used as a positive control for antibacterial activity, while amphotericin B was used as an antifungal positive control.

### Results and Discussion

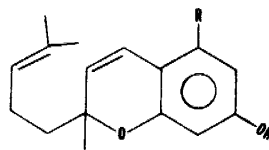
Although cannabichromene is now known to be one of the major cannabinoids in cannabis, little work has been done to investigate its biologic activity. We developed a synthetic procedure to prepare cannabichromene in large quantities<sup>7</sup> in order to study the pharmacology of cannabi-

chromene for possible therapeutic value. This report deals with the antiinflammatory and antimicrobial (both antibacterial and antifungal) effects of cannabichromene. In addition, we investigated the same properties for some of its homologs and isomers whose structures are shown in Fig. 3. The acute  $LD_{50}$  of cannabichromene was determined in mice using two routes of administration. There were no deaths resulting from oral administration at doses up to 3000 mg/kg, while the same dose level given subcutaneously resulted in 10 to 20 per cent mortality.

The antiinflammatory activity of cannabichromene (IV) and its  $C_1$ -homolog (VI) was determined by both the carrageenan-



CBC, R =  $C_5H_{11}$  (IV)  
CBC- $C_1$ , R =  $CH_3$  (V)  
CBC- $C_0$ , R = H (VI)



iso-CBC, R =  $C_5H_{11}$  (VII)  
iso-CBC- $C_0$ , R = H (VIII)

Fig. 3. Cannabichromene (CBC) and its homologs and isomers.

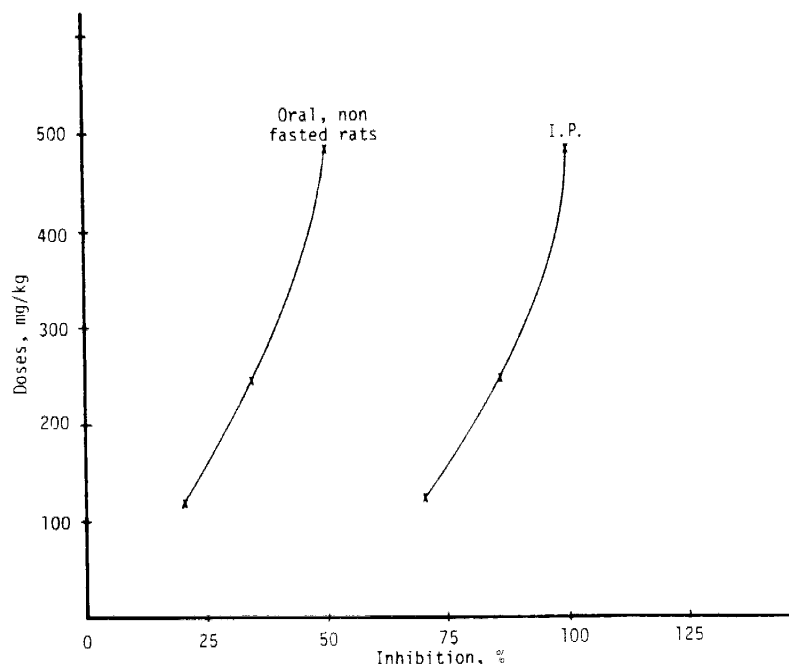


Fig. 4. Dose-response curve for the inhibition of the carrageenan-induced rat paw edema using cannabichromene by both intraperitoneal and oral administration.

induced rat paw edema assay and the erythrocyte membrane stabilization method. Table II shows the activity of both compounds at different dose levels given intraperitoneally and also the activity of cannabichromene given orally to both fasted and nonfasted rats. In each test, comparison was made with phenylbutazone (PBZ). It is clear that cannabichromene was more active by the intraperitoneal route than orally and that the activity was comparable to that of its  $C_1$ -homolog (V) and to PBZ (Table II). Cannabichromene was more active at 240 or 480 mg/kg than was PBZ at 120 mg/kg. PBZ could not be tested at higher doses because of its toxicity. The dose-response curve for the antiinflammatory activity of cannabichromene when given intraperitoneally and orally is given in Fig. 4.

In addition to the previous assay, the antiinflammatory activity of compounds IV and V was confirmed by their inhibition of

the heat-induced hemolysis of erythrocytes. Other homologs and isomers (VI-VIII) were tested using the same assay. Table III gives the per cent inhibition produced by solutions of the different compounds as compared to PBZ and aspirin (ASA). All homologs and isomers were tested at  $1 \times 10^{-4}M$  dilution; however, cannabichromene was tested at different concentrations. The dose-response curve for the inhibition of various concentrations of CBC is shown in Fig. 5. Results utilizing this assay indicated that all compounds tested were significantly more active than PBZ and aspirin. The activity of IV and V indicated by this screen was parallel to their activity in the rat paw edema assay.

It should be concluded that cannabichromene and its homologs and isomers do possess antiinflammatory activity as measured by these two screens. The activity of cannabichromene through the oral route, its safety and its lack of behavioral-type

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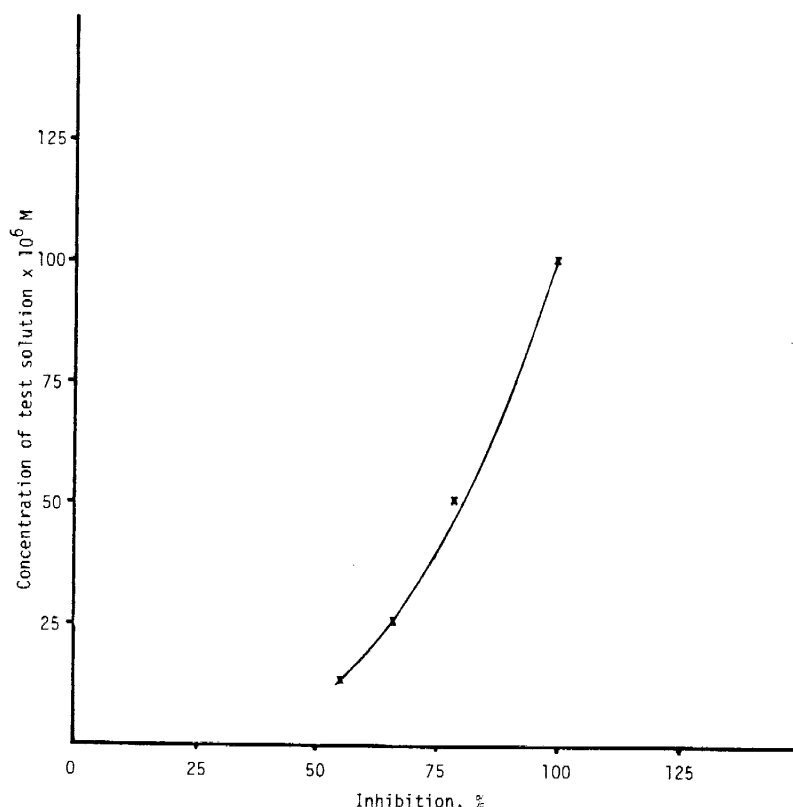


Fig. 5. Dose-response curve for the inhibition of heat-induced erythrocyte hemolysis using different concentrations of cannabichromene.

(psychotomimetic) activity characteristic of THC (I) indicate its therapeutic potential for the treatment of inflammatory diseases. Further testing is under way to ascertain the nature of this potential.

Compounds IV-VIII were subjected to antibacterial and antifungal activity screens in addition to the antiinflammatory activity screens. Organisms utilized in the screens included gram-positive, gram-negative, and acid-fast bacteria as well as different types of fungi (see Table I).

The qualitative screen, the agar well diffusion assay, showed that these compounds possess strong antibacterial and mild antifungal properties. These compounds exhibited large zones of inhibition when compared to positive standards at the same concentrations. The minimum inhibi-

tory concentrations (MIC) for these compounds were determined using selected bacteria and fungi as recorded in Tables IV and V, respectively. The organisms selected for the MIC determination were based on the largest zone of inhibition resulting in the qualitative screen. It is evident from Table IV that cannabichromene (IV) and cannabichromene-C<sub>6</sub> (VI), their isoderivatives (VII and VIII), and cannabichromene-C<sub>1</sub> (V) were more active against *B. subtilis* than streptomycin sulfate. Both IV and V were more active against *S. aureus* than the reference compound. On the other hand, the only homolog with comparable activity to streptomycin against *M. smegmatis* was cannabichromene-C<sub>1</sub>. Although the other homologs and isomers were moderately active, they showed

**TABLE IV**  
**Minimum Inhibitory Concentration (MIC) of**  
**Cannabichromene and Its Homologs and**  
**Isomers Against Different Organisms**

Compound	MIC ( $\mu\text{g/ml}$ )					
	<i>B. subtilis</i>		<i>S. aureus</i>		<i>M. smegmatis</i>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Cannabichromene (IV)	0.39	0.78	1.56	1.56	12.5	25.0
Isocannabichromene (VII)	0.78	3.12	NT	NT	25.0	25.0
Cannabichromene- $\text{C}_6$ (VI)	6.25	12.5	12.5	12.5	12.5	12.5
Streptomycin $\text{SO}_4^*$	6.25	25	3.12	12.5	1.56	1.56
Cannabichromene- $\text{C}_1$ (V)	3.12	3.12	3.12	3.12	3.12	6.25
Isocannabichromene- $\text{C}_6$ (VIII)	6.25	6.25	12.5	12.5	12.5	12.5
Streptomycin $\text{SO}_4^*$	3.12	6.25	6.25	6.25	6.25	6.25

\* Streptomycin  $\text{SO}_4$  was used as standard for tests performed on two different days.

higher MIC values when compared to the standard.

In the antifungal assay (Table V), cannabichromene- $\text{C}_1$  and isocannabichromene- $\text{C}_6$  showed better activity against *T. mentagrophytes* than amphotericin B. All other

compounds had mild to moderate activity against all fungi tested.

The combined antibacterial and antifungal activities of cannabichromene and its homologs and isomers are quite encouraging. Further testing is in progress to assess

**TABLE V**  
**Minimum Inhibitory Concentration (MIC) of**  
**Cannabichromene and Its Homologs and Isomers**  
**Against Different Fungi**

Compound	MIC ( $\mu\text{g/ml}$ )					
	<i>C. albicans</i>		<i>S. cerevisiae</i>		<i>T. mentagrophytes</i>	
	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr
Cannabichromene (IV)	NT	NT	25	50	25	50
Amphotericin B*	NT	NT	3.12	3.12	NT	NT
Isocannabichromene (VII)	50	100	NT	NT	NT	NT
Cannabichromene- $\text{C}_6$ (VI)	50	50	25	25	25	25
Amphotericin B*	1.56	1.56	0.78	0.78	NT	NT
Cannabichromene- $\text{C}_1$ (V)	NT	NT	6.25	12.5	6.25	6.25
Isocannabichromene- $\text{C}_6$ (VIII)	12.5	25	NT	NT	6.25	6.25
Amphotericin B*	1.56	6.25	0.19	0.78	12.5	25

\* Amphotericin B was used as standard for tests performed on three different days.



the activity of these compounds, analogs, and derivatives against other organisms.

### Acknowledgments

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