Diaryl Pyrroles: A New Series of Anti-Inflammatory Agents

In the years since the reports of the anti-inflammatory activity of aspirin, phenylbutazone, fenamates, and indomethacin, there has been an increasing emphasis on the anti-inflammatory activity of some aromatic or heterocyclic compounds of acidic nature, while only little attention if any has been paid to the activity of neutral compounds[1].

In this paper, we wish to describe briefly the anti-inflammatory activity of a new series of non-ionic pyrrole compounds: 2-alkyl-4, 5-diphenyl-pyrroles. They were prepared from 2-alkyl-4, 5-diphenyl-pyrrole-3-carboxylic esters by elimination of the 3-substituents with 80% sulfuric acid.

Anti-inflammatory activity of compounds was examined in a variety of animal tests. Some of the compounds inhibited the edema response in rats to the injected carrageenin[2], and the erythema response in guinea-pigs to the ultraviolet radiation[3]. In the Table the results of paw edema assay in rats are summarized.

Substitution with chloro- or methoxy-group in *para*-position of both the phenyl rings caused a prominent increase in the anti-inflammatory activity. With respect to the substitution with alkyl group in 2-position of the pyrrole ring, the chain length was critical for full activity of the compound, and 2-methyl-4, 5-*bis*(*p*-methoxyphenyl)pyrrole: 'bimetopyrol' was found as the most potent compound among them[4].

The spectrum of anti-inflammatory activity of these compounds was generally in common with that of the other non-steroidal anti-rheumatic drugs. Thus, bimetopyrol suppressed ultraviolet-induced erythema in guinea-pigs (ED_{50} was 9 mg/kg by i.p. injection) and acetic acid-induced writhing in mice, and increased the pain threshold at the yeast-injected paw in rats (13.3 times as potent as aspirin). Also, it prevented the enzyme release from the isolated rat liver lysosomes, as aspirin, phenylbutazone, and flufenamic acid[5].

On the other hand, this compound was not effective on the vascular permeability changes caused by s.c. injection of the mediators such as serotonin or bradykinin, again in agreement with the characteristics of indomethacin and phenylbutazone[6].

The anti-edema activity of the pyrrole compounds was not attributable to the endogenous glucocorticoid, because the activity was not affected by adrenalectomy of the animals used. Bimetopyrol was very effective in minute amount, when it was given to the inflamed paw directly (dose required for 50% inhibition was 1 µg/paw). Furthermore, there was a good correlation between the oral activity and its content in the inflamed tissue which was determined fluorometrically. These findings support the possibility that this compound itself and not the metabolite would act on the target directly at the site of inflammation. As an experimental model of the human chronic and systemic inflammation, we used the adjuvant-induced polyarthritis in rats. In order to get precise potency ratios between phenylbutazone and the two active pyrrole compounds, a single self-contained experiment has been made, specified by the use of well-established arthritic animals and by the selection of doses of drugs to fit the statistical analysis. By 6 points assay, the

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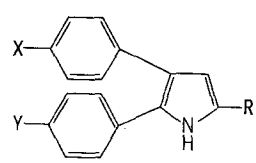
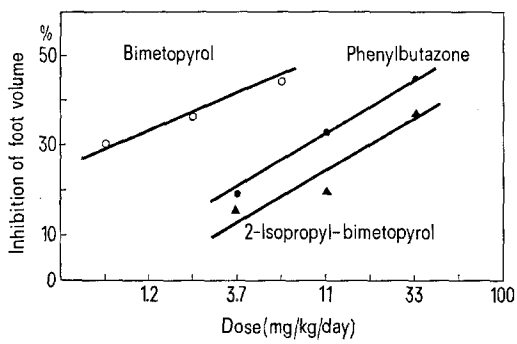


X	Y	R	% Inhibition (100 mg/kg, by mouth)	Dose required for 50% inhibition (mg/kg)
–H	–H		22	
–OH	–OH		1	
$-NHCOCH_3$	$-NHCOCH_3$		9	
–Cl	–Cl		46	
$-OCH_3$	$-OCH_3$	$R = -CH_3$	62	
$-OCH_3$	–H		31	
–H	$-OCH_3$		17	
$-OCH_3$	–Cl		60	
		–H		more than 100
		$-CH_3$		9
		$-C_2H_5$		25
$X = Y = -OCH_3$		–iso C_3H_7		36
		–n C_4H_9		60
		–tert C_4H_9		more than 100
	Phenylbutazone			62

Anti-edema activity of selected pyrroles. Carrageenin (0.05 ml of 1% solution) was injected s.c. into the hind paw 30 min after administration of drug (dissolved in olive oil) by mouth. The effect measured 3 h later.

therapeutic potency of bimetopyrol and its 2-isopropyl analogue were calculated as 8.46 (4.27–17.4) and 0.49 (0.21–0.91) times as potent as phenylbutazone, respectively.

Toxicological investigations in mice have shown that the values of acute LD_{50} of bimetopyrol were 3.6 g/kg (male) and 3.4 g/kg (female) by mouth, and 2.7 g/kg (male) and 1.9 g/kg (female) by i.p. injection. In rats, those were 2.3 g/kg (male) and 1.8 g/kg (female) by mouth. In chronic toxicity tests, rats were dosed daily for 6 months by mouth with 100, 50, 25, and 5 mg/kg. During the course of investigation, 2 of 10 female animals receiving the highest dose died. The death was assumed to result from gastro-intestinal ulceration and systemic peritonitis. At the termination of the experiment, none of the abnormalities were observed in the animals which had received bimetopyrol at doses not more than 25 mg/kg/day, while 5 of 38 animals receiving higher doses had ulcers in stomach or intestine and also there was a slight increase in liver weight. Dogs tolerated daily dose of 150 mg/kg for 3 months without any significant signs of abnormality in pathological examinations.



Curative effect of selected pyrrole compounds on established arthritis in rats. Sprague Dawley female rats were injected intracutaneously in the right hind paw with 0.5 mg of heat-killed *M. butyricum* in 0.05 ml of mineral oil. 18 days after adjuvant injection, the well-established arthritic animals were selected and subjected to the 7 days therapy with drugs. The mean value of the increased foot volume at 18th day after injection was 4.31±0.11 ml. 3 doses for each drug and 7 animals for each group were used. Drugs suspended in aqueous tragacanth were given orally, and the foot volume of right hind paw of each animal was measured before and after the therapy.

Zusammenfassung. Die antiinflammatorischen Eigenschaften von verschiedenen Diaryl-pyrrol-Derivaten wurden untersucht. Es wurde festgestellt, dass eine repräsentative Verbindung, 2-Methyl-4, 5-*bis*(*p*-metoxyphenyl)-pyrrol, eine ausgeprägte Hemmung gegen das akute Ödem und die chronische Arthritis der Ratte zeigt.

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Abnormalities of Lung Lipids Following Inhalation of Quartz

After inhalation of quartz for extended periods and prolonged survival thereafter, specific pathogen-free (SPF) rats failed to acquire typical silicotic lesions but instead responded by the development of pulmonary alveolar lipo-proteinosis[1]. Analysis of this condition by histochemical, chemical and immunological means established that, apart from the presence of quartz, the experimental disorder was essentially similar to the human disease[2]. The same samples of quartz were shown to be fibrogenic by intraperitoneal and intratracheal injection into other rats, both conventional and SPF. The outstanding feature of experimental lipo-proteinosis was the large lipid component of the alveolar material and histochemically both neutral lipids and phospholipids were identified. Ultrastructural observations on the same material[3] demonstrated extensive alveolar accumulation of phospholipid structures which assumed several morphological forms characteristic of the liquid-crystalline phase. However, the earlier chemical procedures did not distinguish particular groups of lipids and we now report a refined analysis, indicating implications of the quantitative and qualitative changes.

Methods. Male SPF rats were exposed for 8 or 26 weeks to the inhalation of Minusil quartz at an atmospheric concentration of 37 or 12 mg/m³ and survived subsequently for 20 or 30 weeks. The lungs were quickly removed and frozen in liquid nitrogen. They were sealed in plastic tubes and remained frozen under nitrogen until required for chemical fractionation. The biochemical procedures employed have been described elsewhere[4]. In other rats exposed to quartz simultaneously it was confirmed that the histological changes were typically those of alveolar lipo-proteinosis and not of silicosis.

Results. To demonstrate the overall directions of change, the experimental animals are compared with control rats

Table I. Quantitative changes in lipid categories

	Control Mean (mg)	Experimental Mean (mg)	Increase factor
Total lipids	73	1605	22
Neutral lipids (including cholesterol)	27.1	232	8
Cholesterol	6.1	106	17
Phospholipids			
Total	35.6	1210	34
Lecithins	19.1	1056	55
Dipalmityl lecithin	10.9	699	64
Phosphatidylethanolamines	7.7	70	9
Sphingomyelins	4.7	35	7

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