Table I—Typical Response Factors of Creatinine at Various Spiked Concentrations in Saliva Samples

Sample	Spiked Concentration, mg %	Peak Height[a], cm	Response Factor[b]
1	0	1.16	—
2	0.05	3.04	37.60
3	0.10	5.12	39.60
4	0.20	9.10	39.70
5	0.30	12.70	38.47
6	0.50	19.60	36.88
Average ± *SD*			38.45 ± 1.232

[a] The detector sensitivity was set at 0.005 *A* full scale, and a 25.4-cm recorder chart paper was used. [b] Response factor = (peak height from spiked sample—peak height from unspiked sample)/spiked creatinine concentration.

(Fig. 1). The creatinine peak was not as well resolved as that observed in plasma or serum samples using simple deproteinization (5).

Methylene chloride in the sample preparation served two purposes. It removed interfering substances from saliva through extraction. It also concentrated the aqueous supernate as compared to the previous deproteinization procedure (5), since essentially all acetonitrile remained with the methylene chloride layer in the last step. Our preliminary study indicated that most creatinine remains in the upper aqueous layer. In other words, about a threefold increase in sensitivity can be achieved by the present extraction method.

In our previous study (5), a variable wavelength detector set at 215 nm was used for creatinine quantitation. Although creatinine absorption at 254 nm in the mobile phase used here was only ~28% of that at 215 nm, the higher signal-to-noise ratio using the 254-nm fixed wavelength detector as compared to the variable wavelength detector (7) would still make the former detector more sensitive. Based on a criterion of a signal-to-noise ratio of 3, one could estimate the lower limit for quantitation with the present method as 0.0065 mg %.

The satisfactory creatinine peak height linearity with increasing concentrations was demonstrated by the response factor studies (Table I). The coefficient of variation in the range studied was 3.2%. The coefficients of variation for the intraassay and interassay studies (n = 6) using a saliva sample with 0.048 mg % creatinine were 4.2 and 6.7%, respectively. The coefficients of variation were reduced to 2 and 5.5%, respectively, with a 0.248-mg % saliva creatinine sample. Over several months, many hundred saliva samples have been satisfactorily analyzed by the present method.

In most of our saliva creatinine studies, the recorder chart speed was 10 cm/hr. The advantages of such a slow chart speed were discussed previously (8). In the present study, a micrometer[5] with its dial graduated in 0.1-mm increments was used for the measurement of peak heights.

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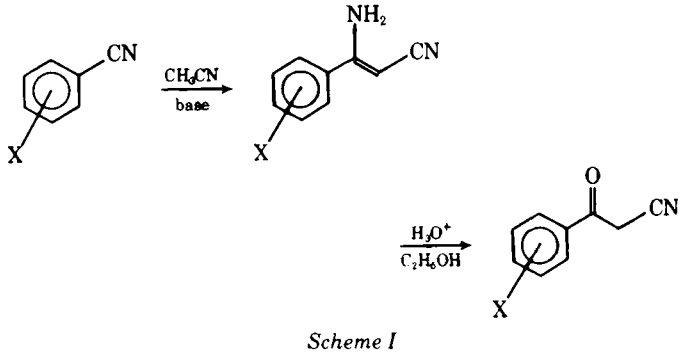
Potential Antiarthritic Agents I: Benzoylacetonitriles

Keyphrases □ Antiarthritic agents—benzoylacetonitriles, synthesis, screened in rats, structure–activity relationships □ Benzoylacetonitriles—antiarthritis activity, synthesis, screened in rats, structure–activity relationships

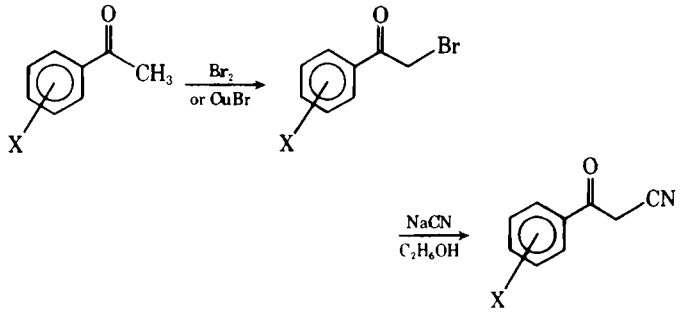
To the Editor:

Rheumatoid arthritis, a chronic inflammatory disorder of unknown etiology, is treated largely with agents useful only in ameliorating the acute inflammatory symptoms, *i.e.*, pain and swelling. Clearly, nontoxic drugs effective in the remission of joint and cartilage destruction are critically needed. We screened compounds in the chronic Freund's adjuvant-induced arthritis model (1) and discovered that benzoylacetonitrile (I) and its monofluorophenyl analogs (II–IV) were highly effective in this assay.

Concurrently, we found this series to be inactive against carrageenan-induced edema in rats and urate synovitis in dogs and only weakly active in suppressing UV-induced erythema in guinea pigs. When tested for the suppression of prostaglandin synthesis *in vitro*, benzoylacetonitrile had only one-third of the potency of aspirin. Additionally, I and IV displayed little or no ulcerogenic potential in rats at total daily doses as high as 800 mg/kg. These facts suggest that prostaglandin synthesis inhibition is unlikely to ac-



Scheme I



Scheme II

count for this striking suppression of developing adjuvant arthritis.

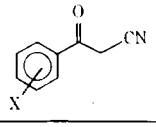
A series of phenyl-substituted benzoylacetonitriles was prepared and screened for activity in the above-mentioned assays. These analogs were easily obtained, as in Scheme I, *via* the β-aminocinnamonitriles (2) followed by acid hydrolysis.

The most useful conditions for enamine intermediate preparation employed 1 equivalent of sodium hydride in refluxing ether with 0.1 equivalent of *tert*-butyl alcohol. Alternatively, benzoylacetonitriles were prepared by cyanide displacement on a phenacyl bromide (3) as in Scheme II. Table I lists some representative benzoylacetonitriles from the many prepared in this investigation.

The compounds listed in Table I were screened in the rat adjuvant arthritis assay as described previously (1). Measurements of swelling and weight gain were made 14 days following challenge with Freund's adjuvant. Compounds that produced a statistically significant percent inhibition of the control swelling were accepted as active. Only benzoylacetonitrile (I) and its fluoro analogs (II–IV) displayed activity in this model.

Table II presents dose–response data for I and IV on adjuvant arthritis in rats. These compounds showed marked suppression of both primary and secondary lesions. Additionally, they suppressed both lesions for at least 7 days after cessation of treatment.

Table I—Benzoylacetonitrile Analogs



Compound	X	Method	Yield, %	Melting Point	Reference
I[a]	H	—	—	79–80°	4 (mp 80–81°)
II	2-F	I	87	53–54°	5 (mp 53°)
III	3-F	II	37	69–70°	6
IV	4-F	I	80	78–80°	6
V	2-Cl	I	77	50–54°	7 (mp 56–57°)
VI	4-Cl	I	53	126–130°	3 (mp 128°)
VII	3-Br	I	88	93–95°	7 (mp 88–89°)
VIII	4-Br	II	42	164–165°	3 (mp 158°), 7 (mp 160–161°)
IX	4-CH_3	II	40	106–106.5°	3 (mp 102°)
X	4-OH[b]	II	38	168–172° dec.	5, 7 (mp 182–183°)
XI[a]	4-NH_2	—	—	157–160°	7 (mp 157–158°)

[a] Obtained from Aldrich Chemical Co. [b] Elemental analysis acceptable to within 0.4% for $C_9H_7NO_2 \cdot 1/4\ H_2O$.

Table II—Effect of Benzoylacetonitrile (I) and *p*-Fluorobenzoylacetonitrile (IV) on Developing Adjuvant Arthritis in Rats (Pooled Data)[a]

Compound	Oral Dose, mg/kg	Number of Animals	Mean Weight Gain, g	Inhibition of Swelling, % Primary Lesion	Inhibition of Swelling, % Secondary Lesion
Normal rats	—	186	77	—	—
Adjuvant controls		630	36	0	0
Indomethacin	4		Toxic		
	2	57	68	51	38
	1	54	63	46	34
	0.5	54	53	40	25
I	400	18	31*	81	84
	200	18	56	73	70
	100	36	49	63	70
	50	54	53	51	52
	25	36	54	54	40
	12.5	18	49	37	43
IV	400	18	42*	75	72
	200	18	67	52	63
	100	18	63	46	44
	50	36	61	56	13
	25	18	55*	33	25
	12.5	18	62	34	22

[a] All values in the treated groups, other than those marked with an asterisk, were significantly different from the adjuvant arthritic controls; $p < 0.05$ by t test.

Analogs II and III also were effective in the rat adjuvant arthritis assay and were inactive when tested in the prostaglandin-mediated assays (*i.e.*, rat carrageenan edema and guinea pig UV erythema).

We concluded that these agents (I–IV) act by a pharmacological mechanism different from that of the classical aspirin-like anti-inflammatory drugs and have a unique potential as structurally novel agents for antiarthritic therapy. Initial animal studies suggest that reticuloendothelial stimulation may be one possible mechanism of action. A detailed discussion of the chemical and pharmacological aspects of this work will be published.

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