Table 1. COMPARISON OF TECHNIQUES FOR ISOLATION OF T-MYCOPLASMAS FROM HUMAN GENITAL SPECIMENS

Specimens examined	No. of isolations as determined by: Colonies on solid medium	Colour change in Liquid medium	Colour change in Solid medium	Colonies alone	Colour in liquid alone	Total positive
Number 38	10	15*	7	1†	6	16
Percentage 100	26	40	18	3	16	42

* Confirmed by subsequent subculture in which colour change observed in liquid medium and colonies observed on solid medium.

† Unconfirmed; that is, failure to subculture.

cause a change of colour[9]. The surface of solid medium was examined with a "plate" microscope at × 60-120 magnification. The use of diffuse transmitted light was an important factor in detecting colonies.

Urethral swabs were broken off into 2·0 ml. of basic liquid medium. A portion of this was transported in crushed ice to Salisbury the same day. In both laboratories 0·1 ml. amounts were inoculated into the liquid medium containing urea, from which two further ten-fold dilutions were made; 0·1 ml. amounts were also inoculated onto the solid medium with and without additives. The cultures were incubated at 37° C, the solid media in a humidified atmosphere of 95 per cent (v/v) N_2 and 5 per cent (v/v) CO_2. The *p*H of liquid medium in those cultures which changed colour increased at least 1 *p*H unit. Results of tests with liquid and solid media were recorded independently. Isolation results from each laboratory were closely similar. As judged by detection of colonies, 26 per cent of the specimens contained T-mycoplasmas (Table 1). Forty per cent of the specimens, however, produced a change of colour in liquid medium; in each of these cases a change of colour occurred on subculture to liquid medium and colonies developed on subculture to solid medium. One specimen only seemed to produce colonies without causing a change of colour in liquid medium, but colonies could not be subcultured. It seems therefore unlikely that there are T-mycoplasmas which produce colonies but do not metabolize urea. The occurrence of a change of colour in solid medium was the least sensitive technique. Although colonies were, however, sometimes present without a change of colour, the addition of urea and phenol red is a worthwhile procedure, for a change in colour clearly indicates that colonies are present, and these often have a darker, more granular appearance and are therefore more easily seen[9].

Incubation in N_2 with 5 per cent CO_2 is suitable for colony development if the *p*H of the solid medium is 6·5 or less. If the *p*H is higher (for example, 7·8), a greater concentration of CO_2 (for example, 20 per cent) is required.

The change of colour in liquid medium begins at the bottom of the vial, usually after incubation for 24–36 h, but occasionally after a much longer incubation period; for example, 10 days[10,11]. In all cases we have observed a rapid change of colour on subculture, suggesting that any original slowness was due to only a few organisms being present and/or the presence of a growth inhibitor. The organisms die rapidly at 37° C and so it is important to subculture, chill to 4° C, or store at −20° C or below, immediately a change of colour has occurred.

Spurious changes of colour in liquid medium containing urea may result from several causes: (i) Lack of an air-tight seal to the glass vial. (ii) Contamination with bacteria which metabolize urea—the medium becoming turbid, which is never so with growth of T-mycoplasmas. (iii) Epithelial cells which apparently sometimes contain a urease. This produces a change of colour within a few hours at the bottom of the vial where the cells have settled, but subculture is not followed by a change of colour. We have observed this with specimens from the human pharynx and from the preputial sacs of bulls. (iv) Large-colony-forming mycoplasmas, for example *M. hominis*, which break down arginine to ammonia. The growth of these may on occasion result in a slowly developing, moderate, alkaline change of colour from *p*H 7·0 to 7·3.

Serial ten-fold dilution of clinical specimens in liquid medium, rather than inoculation of a single vial, is valuable because: (i) Medium that changes colour after inoculation with a high dilution of a specimen containing many cells may be used to produce a relatively cell-free agar subculture. (ii) Medium containing a low dilution of a specimen may change colour at a time inconvenient for subculture, and a further opportunity for this may be provided by the higher dilutions. (iii) If changes of colour occur in several vials which contain serial dilutions of a specimen, it is unlikely that they are spurious due to unsealed vials. (iv) Occasionally, medium which contains high dilutions of a specimen changes colour, but it does not with low dilutions. On rare occasions this is caused by the presence of very many organisms, the mechanism of inhibition not being known. More frequently it is caused by an inhibitor of mycoplasma growth in the specimen. We have found such an inhibitor in bovine semen. Sometimes, apparent inhibition of growth occurs when specimens are tested at low dilution, because numerous epithelial and other cells cause acidity of the medium which masks the alkalinity produced by T-mycoplasmas. (v) Dilution of a clinical specimen provides a quantitative measurement of the number of T-mycoplasma organisms present.

In summary, liquid medium containing urea and phenol red affords the most sensitive, quickest and simplest method for isolating T-mycoplasmas. It has been used to isolate them from human materials, from cattle[11–13], and recently from squirrel monkeys and dogs (unpublished results of D. T.-R.).

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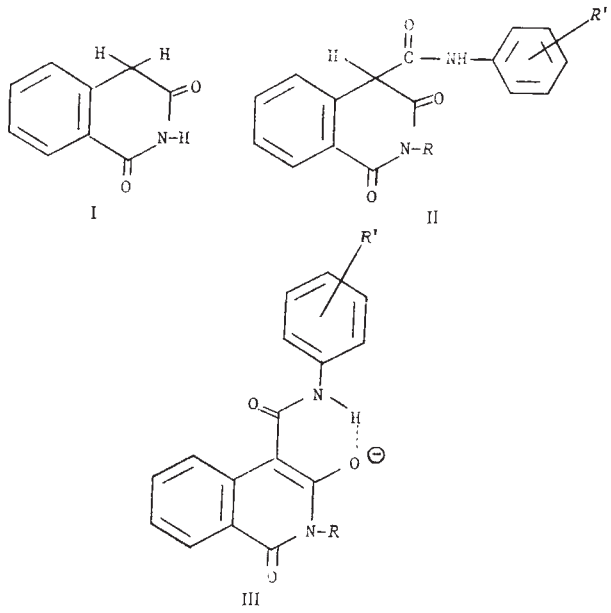
Dioxoisoquinoline-4-carboxanilides—A New Class of Non-steroidal Anti-inflammatory Agents

STRUCTURAL parameters of different groups of non-steroidal anti-inflammatory agents (pyrazolidinediones[1], fenamates[2], indoles[3], oxazoles[4] and thiazoles[4]) have certain similarities which include the presence of a relatively non-basic nitrogen, an acidic (carboxylic or enolic) function and an aromatic moiety. We report here the synthesis and biological properties of a new and potent series of anti-inflammatory agents which manifest similar structural features.



Isoquinoline-1,3(2H,4H)-dione (I), or substituted isoquinoline-1,3-(2H,4H)-diones treated with aryl isocyanates in the presence of a base, give (on aqueous, acidic work-up) fair to good yields of the desired 1,3(2H,4H)-dioxoisoquinoline-4-carboxanilides (II). The infrared and nuclear magnetic resonance spectra of these substances agree with their structures, which contain a non-basic nitrogen and display unexpectedly great acidic properties (pKa' values ~4–6 in 1 : 2 aqueous dioxane). The latter may partially be ascribed to a hydrogen bonded stabilization of the planar, enolate anion (III) which results from the ionization of (II) because a plot of the pKa' values of twenty ortho, meta and para substituted anilides of structure (II) versus the pKa values of the correspondingly substituted anilines yields a straight line having a slope of 0·55 (r = 0·98). This indicates that the effect of R′ on the pKa' values of (II) is proportional to its effect on the electron density about the nitrogen of the related anilines. Some compounds were prepared by treatment of ethyl 1,3(2H,-4H)-dioxoisoquinoline-4-carboxylate with the appropriate aniline in refluxing xylene.

The 1,3(2H,4H)-dioxoisoquinoline-4-carboxanilides show potent anti-inflammatory activity: in both normal and bilaterally adrenalectomized rats they considerably inhibited paw oedema induced by carrageenin[5]; in guinea-pigs the erythemic response to ultraviolet irradiation[6] was inhibited by pretreatment with these compounds; and in more chronic situations, anti-inflammatory activity in the rat was noted both by suppression of cotton string induced granuloma formation and by amelioration of the symptoms of arthritis induced by adjuvant[7]. Chronic administration (50 mg/kg for 30–90 days) of (II*b*) to rats and dogs and of (II*c*) to rats and monkeys induced neither gross nor pathological changes.

Table 1. RELATIVE ANTIOEDEMA POTENCIES (RAT) AND AVERAGE PLASMA HALF LIVES (DOG AND MAN) OF 1,3-(2H,4H)-DIOXOISOQUINOLINE-4-CARBOXANILIDES

Compound	R	R′	Relative potency*	Plasma half life (h) Dog†	Man
(II*a*)	H	H	0·2	2	
(II*b*)	H	*p*-Cl	0·8	19	22‡
(II*c*)	CH_3	H	1·4	6	8‡
(II*d*)	CH_3	*p*-Cl	3·0	10	
(II*e*)	CH_3	*p*-F	2·5	12	
(II*f*)	CH_3	*p*-OH	<0·1	0·2	15§
Phenylbutazone			1·0	6	72[8]

* Oedema induced by subplantar administration of carrageenin to the rat; drugs administered orally 1 h before, and oedema measurement 3 h after, injection of carrageenin.

† Drugs administered intravenously (10–15 mg/kg); assay of drug in plasma samples by extraction and measurement of optical density at 235, 295 and 375 mmicrons in a Beckman *DU* spectrophotometer.

‡ Estimated from decay of plasma concentrations in normal volunteers, after oral administration of a single dose of 1 g. Drug assay as above.

§ Estimated from decay of plasma concentrations following oral administration of a single 1 g dose of (II*c*). Assayed differentially from (II*c*) as above.

Anti-inflammatory potency and plasma half life were both favourably related to the introduction of electronegative substituents in the para position of the carboxanilide moiety (Table 1). Although these effects in part reflect an increase in lipophilicity, an equally important factor appears to be in blocking of the metabolically vulnerable para position. The para hydroxy derivative (II*f*), the chief metabolite of (II*c*) in both animals and man, was essentially devoid of activity in the rat-foot oedema procedure. Further, in view of its extremely short plasma half life in animals, (II*f*) would not be expected to contribute to the anti-inflammatory activity of the parent drug.

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Interaction of Artificial Phospholipid Membranes with Isolated Polymorphonuclear Leucocytic Granules

AFTER the engulfment of particles by rabbit polymorphonuclear (PMN) leucocytes, the membrane of granulocytic lysosomes fuses with the membrane of the phagocytic vacuole, thereby allowing lysosomal enzymes access to the ingested material[1]. To try to understand this specific interaction between lysosomal and vacuolar membranes, we have studied a cell-free system containing isolated PMN granules with artificial model membranes substituted for the vacuolar membrane.

Granules were prepared from PMN leucocytes obtained from sterile, glycogen-induced rabbit peritoneal exudates[2,3]. Artificial phospholipid membranes (spherules) were composed of chromatographically pure l-alpha lecithin, sodium dicetyl phosphate and cholesterol[4]. A second type of phospholipid spherule was prepared by swelling choline-free inositol phosphatide in water for 1 h. Methods described elsewhere were used for determination of phosphorus content of phospholipid spherules[5], beta glucuronidase[6] and beta N-acetylglucosaminidase[7].

The experimental system contained the granule suspension obtained from approximately 7×10^7 cells and the swollen phospholipids. The mixture was buffered to *p*H 5·0 with 0·05 M acetate buffer or 0·05 M citrate–HCl buffer. The final sucrose concentration was 0·25 M. The results were expressed as the percentage of total enzyme activity which was accepted as the amount of enzyme found in an aliquot of granules exposed to 0·01 per cent 'Triton *X*-100'. For electron microscopy the centrifuged pellets (20,000*g*) were fixed at *p*H 7·3 in cold 1 per cent