# PAPER CHROMATOGRAPHY AND CHEMICAL STRUCTURE

## V. TAUTOMERISM. THE DETERMINATION OF TAUTOMERIC EQUILIBRIUM BY PAPER CHROMATOGRAPHY. THIENOL AND *p*-NITROSOPHENOLS

J. GREEN AND S. MARCINKIEWICZ
*Walton Oaks Experimental Station, Vitamins Ltd., Tadworth, Surrey (Great Britain)*

(Received June 26th, 1962)

### INTRODUCTION

If the introduction of a group X into a compound produces a new structure that can exist in tautomeric forms, its effect on $R_M$ will be different from the homologous increment $\Delta R_M(X)$.

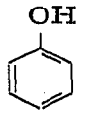
Tautomerism is possible in many types of compound, although its existence is often difficult to establish with certainty. It is most clearly established when both tautomers are sufficiently stable to be capable of independent isolation. Several classic cases of keto–enol and nitroso–oxime tautomerism fall into this category. In certain instances—as with 1,4-naphthaquinol, described below—the individual tautomers are exceptionally stable and, chromatographically, can be dealt with as two separate species; but, in other compounds, interconversion is more rapid and can be expected to occur during chromatography. Most compounds in which tautomerism is postulated are not in fact separable into two forms at all, and the tautomerism is implied by either chemical or physical evidence, sometimes both. (Chemical and physical evidence often fail to agree as to the existence of tautomerism: sometimes they agree but lead to diametrically opposed conclusions as to the nature of the tautomeric form; *cf.* the conflicting evidence as to keto–enol forms in phloroglucinol, and HODGSON[1,2] and ANDERSON AND YANKE[3] on the structure of *p*-nitrosophenol.)

The problem of tautomerism cannot be neglected in any discussion on the relation between chromatography and chemical structure. It is, in many ways, a more complex problem than other "constitutive" effects on $R_M$. Indeed, it is not strictly speaking a constitutive effect at all, since if a compound is tautomeric it must be treated from the theoretical point of view as a mixture of two compounds, in each of which additional constitutive effects on $R_M$(hydrogen bonding, steric and polar interaction) may occur. There are three reasons why tautomerism may be of especial importance in chromatographic studies. Since tautomerism in most cases involves the mobility of a hydrogen atom the two forms can be expected to differ chromatographically to a large extent. Secondly, chromatographic separation and identification is particularly valuable in the study of complex molecules, precisely those in which the possibility of tautomerism is increased and in which tautomers may have increased stability because of increased possibilities of resonance stabilisation. Finally, tautomeric equilibrium is

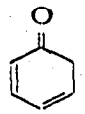
markedly affected by solvents: for example, ethyl acetoacetate contains 0.4 % of enol form in water, but 46.4 % in hexane[4]. Therefore, under the conditions of partition chromatography, providing interconversion of tautomers can take place rapidly enough, each phase may contain the two tautomeric forms in different proportions.

## THE NATURE OF TAUTOMERISM IN PHENOLS AND *p*-NITROSOPHENOLS

The problem of tautomerism in phenols has been reviewed by THOMSON[5]. The chemical and physical properties of phenol itself indicate that it exists almost exclusively in the enol form (I), although the existence of a minute proportion of keto form (II) cannot be ruled out.

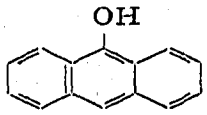



(I)

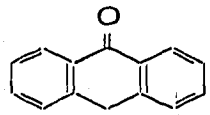



(II)

Although a keto form is intrinsically more stable than an enol form by about 18 kcal/mole, the energetic advantage in ketonisation of (I) is more than offset by the loss in resonance energy involved, about 35 kcal/mole. Polyhydroxybenzenes can be expected to contain a greater proportion of keto forms, since in them the gain in energy on ketonisation may more nearly compensate for the loss of resonance energy. Nevertheless, although in the most favourable compounds, such as phloroglucinol, there is some chemical evidence for ketone formation, the existence of such forms in the ground state is highly speculative. Bicyclic and polycyclic phenols show greater tendency to ketonisation, since the loss of resonance energy involved becomes progressively smaller as the number of aromatic rings increases. Thus, with 9-anthranol (III), the loss of resonance energy in converting the central "aromatic" ring to a quinonoid structure is so small (about 12 kcal/mole) that the keto form is energetically favoured and this indeed is the simplest carbocyclic monophenol to exist in both tautomeric forms, the keto form being 9-anthrone (IV).



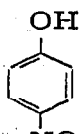

(III)



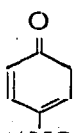

(IV)

In polycyclic dihydroxy compounds ketonisation is still more favourable energetically, and the bicyclic compound, 1,4-naphthaquinol exists in a stable diketo form.

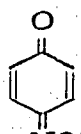
The problem of tautomerism in nitrosophenols is also still open. Theoretically *p*-nitrosophenols can exist in three forms (V), VI) and (VII), although form (VI) is usually ignored.




(V)




(VI)




(VII)

Chemical evidence indicates tautomerism in these compounds, while the physical evidence is inconclusive. HODGSON[1,2] has amassed strong chemical and physical evidence in favour of the nitroso form as the chief component. ANDERSON AND YANKE[3], from a spectral study, concluded that *p*-nitrosophenol existed as an oxime (VII), but their interpretation of the evidence has been critized by HODGSON[2]. More recently SCHORS, KRAAIJEVELD AND HAVINGA[6] have re-examined the spectra and estimated that *p*-nitrosophenol contains about 20 % of (V) in methanol and 30 % in acetone. 4-Nitrosonaphthol, on the other hand, exists exclusively as oxime form (VII) owing to the stability of the naphthaquinone structure. There is no evidence, either chemical or physical, for the existence of a nitroso form in this compound[7].

In order to study tautomerism chromatographically it is essential to eliminate (or to be able to account for) all other molecular factors influencing $R_M$. This is usually a difficult problem. Thus resorcinol and hydroquinone, in both of which the possibility of limited tautomerism exists, separate chromatographically[8]; but the effect of tautomerism in these molecules (if it exists) cannot be studied without taking into account the general problem of the separation of *m*- and *p*-isomers[9]. We have therefore limited ourselves here to a study of tautomerism in (a) the one monohydric phenol in which it has been shown unequivocally to occur and in which other constitutive effects on chromatography are non-existent and (b) some *p*-nitrosophenols, whose tautomerism is well established also.

## THEORETICAL

The chromatographic behaviour of a tautomeric mixture theoretically depends on the rate of interconversion of the tautomers and on the position of the tautomeric equilibrium. If the position of equilibrium is such that one tautomer exists only to a small extent, the substance will, from the practical point of view, behave as if it were a single form. If sufficient of a second form is present to affect $R_M$, chromatographic behaviour will be governed by the kinetics of interconversion. If the rate is slow, as in 1,4-dihydroxynaphthalene or naphtharesorcinol[5], the two tautomers should be separable and their individual $R_M$ values can be calculated from MARTIN's equation, with due consideration to other constitutive effects (see the separation of the two forms of 1,4-naphthaquinol, described below). When the rate of interconversion is more rapid, however, the tautomeric change may interfere with the establishment of chromatographic equilibrium and, consequently, the solute will migrate, not as a zone, but as a diffuse streak. Such a compound is truly impossible to chromatograph in any system in which such rates occur. (We have found what appears to be a genuine example in *o*-nitroso-*p*-chlorophenol. On reversed phase chromatography with olive oil as stationary phase and aqueous ethanol as mobile phase, it gives only elongated streaks, although it is readily soluble in both phases. The compound was also impossible to chromatograph in direct phase trigol/ether systems although once again it was soluble in both phases. The effect could not have been due to adsorption as other nitrosophenols could be run satisfactorily in these systems.) Finally, when the interconversion of tautomers is very rapid, the solute will migrate as a discrete spot and its $R_M$ value will not be the theoretical $R_M$ value of either tautomer but will be determined by the relative amounts of each form in each phase.

The effect of tautomerism on $R_M$ can be studied by the following treatment,

which also makes it possible to determine the position of tautomeric equilibrium. Consider two tautomeric forms, A and B, of a compound. Let $x$ and $y$ be the concentration of form A and $z$ and $u$ the concentration of form B in the stationary and mobile phases respectively, during the development of the chromatogram. The condition for chromatographic migration as a discrete zone is that all these concentrations are at equilibrium. According to CONSDEN, GORDON AND MARTIN[10]

$$\alpha = \frac{A_L}{A_S}\left(\frac{1}{R_F} - 1\right) \tag{1}$$

where $\alpha$ is the partition coefficient of the solute and $A_L$ and $A_S$ are the cross-sectional areas of the moving and stationary phases respectively. Then, at equilibrium,

$$\frac{x}{y} = \frac{A_L}{A_S}\left(\frac{1}{R_{F(x,y)}} - 1\right) \tag{2}$$

$$\frac{z}{u} = \frac{A_L}{A_S}\left(\frac{1}{R_{F(z,u)}} - 1\right) \tag{3}$$

and

$$\frac{x+z}{y+u} = \frac{A_L}{A_S}\left(\frac{1}{R_{F(\exp)}} - 1\right) \tag{4}$$

where $R_{F(\exp)}$ = the experimentally found $R_F$ value of the tautomeric compound, $R_{F(x,y)}$ = the hypothetical $R_F$ value of A, and $R_{F(z,u)}$ = the hypothetical $R_F$ value of B.

By rearranging and combining eqns. (2), (3) and (4), we obtain the following expression for the position of the equilibria.

In the stationary phase,

$$\frac{x}{z} = \left[\frac{\frac{1}{R_{F(x,y)}} - 1}{\frac{1}{R_{F(z,u)}} - 1}\right]\left[\frac{\frac{1}{R_{F(z,u)}} - \frac{1}{R_{F(\exp)}}}{\frac{1}{R_{F(\exp)}} - \frac{1}{R_{F(x,y)}}}\right] \tag{5}$$

In the mobile phase,

$$\frac{y}{u} = \frac{\frac{1}{R_{F(z,u)}} - \frac{1}{R_{F(\exp)}}}{\frac{1}{R_{F(\exp)}} - \frac{1}{R_{F(x,y)}}} \tag{6}$$

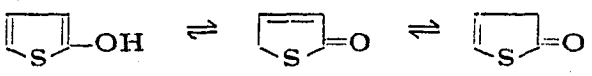
The solvent effect on the tautomeric equilibrium is given by the empirical equation of DIMROTH[11], which was verified by MEYER[12]:

$$\frac{C_A}{C_B} = \frac{L_A}{L_B} \cdot G$$

where $C_A$ and $C_B$ are the concentrations of A and B and $L_A$ and $L_B$ their solubilities, respectively. $G$ is a constant, which is characteristic for the system and is independent of the nature of the solvent.

This expression indicates that tautomeric equilibria can be studied chromatographically when the ratios of the solubilities of A and B in the stationary and mobile phases are considerably different, as in the case of systems such as keto–enol, but not when the solubility ratios are similar.

If the hypothetical $R_F$ value[8] for the two forms can be calculated by means of the group additivity principle, the position of tautomeric equilibrium can be calculated from the experimental $R_F$ value of the tautomeric compound. For this study we have used the heterocyclic phenol, thienol (2-hydroxythiophen) (VIII).



(VIII)

This substance was recently prepared by HURD AND KREUZ[13] who offered clear chemical and physical evidence for its existence in keto and enol forms. There are, as shown, two keto forms possible, the 3- and 2-butenolactones; but the ultra-violet absorption spectrum indicates that the 2-butenolactone form is predominant, and in the following calculations we have assumed that only this keto form is present. Thienol is the only known monohydric mononuclear phenol that has been unequivocally shown to be tautomeric. This is due to the markedly diminished aromatic character of the thiophen ring.

The tautomeric equilibrium was also determined in *p*-nitrosophenol and 4-nitroso-1-naphthol.

## EXPERIMENTAL

### *Compounds*

With the exception of *p*-(2-thiophenylmethoxy)-phenol, all the compounds are known and were either obtained commercially or prepared by literature methods.

*p-(2-Thiophenylmethoxy)-phenol* was prepared by heating hydroquinone (20 g), sodium (1 g), chloromethylthiophen (5 g) and ethanol (250 ml) under reflux for 3 h. The product was extracted into ether, washed with water and then chromatographed in benzene on 50 g of Decalso F. Elution with benzene gave a fraction that coupled with diazotized *o*-dianisidine to give a red-brown colour. Crystallisation from light petroleum–benzene gave the required product as white crystals, m.p. 100–101°. It analysed correctly.

### *Chromatography*

Whatman No. 4 papers (23 × 57 cm) were impregnated with trigol (triethylene glycol, 10 % in chloroform) and dried for 1 h. The mobile phase was either (A) di-isopropyl ether or (B) di-*n*-butyl ether, the ether being saturated with trigol. Chromatograms were developed by the descending method in an atmosphere of nitrogen. Solutes were spotted as thin lines 3 cm long, 60 $\mu$g of each substance being used. Development times of 1 h, 2 h, and 4 h were used, giving solvent front migrations of 21, 32 and 46 cm respectively. The $R_F$ values were shown to be independent of the development time. Some of the key compounds (such as *p*-benzoquinone and *p*-nitrosoanisole) were relatively volatile and were chromatographed by a special technique. The paper was placed between two glass sheets (56 × 10 × 0.5 cm), so that the starting line was between the sheets and the end of the paper dipped into the solvent trough. Chroma-

tography was carried out horizontally. (This technique is a modification of our tankless method, previously described[14], and is suitable if the solvents are relatively non-volatile and anhydrous and only a few compounds are to be chromatographed. As pointed out earlier, glass sheets cannot be used in a pile, as they distort under pressure.) $R_F$ values for standard substances were found to be the same by the two techniques.

*Over-running*

To obtain the necessary information on tautomerism it is often necessary to chromatograph substances with widely differing speeds of migration. Thus, quinol migrates very slowly in the trigol/dibutyl ether system. To obtain the $R_F$ value of this compound accurately, therefore, the over-running technique was used. Quinol was spotted side by side with a marker substance of known $R_F$, *p*-benzoquinone dioxime, and the chromatogram developed by the descending method for 28 h. During this time the solvent over-ran the paper and the marker substance had migrated nearly the whole length of the sheet. Since $R_F$ was shown to be independent of development time, the $R_F$(quinol) could be determined from the $R_F$ value of the marker.

*Spot visualization*

(a) Hydroquinones and their mono-ethers with a mixture of equal volumes of 0.2 % ethanolic ferric chloride and 0.5 % ethanolic 2,2-dipyridyl. (b) Phenols and oximes with 5 % aqueous $K_2CO_3$ followed by diazotised *o*-dianisidine. (c) Quinones with 50 % aqueous ethylamine. (d) *p*-Nitrosoanisole with a 20 % (v/v) solution of aniline in acetic acid. (e) Dihydro-1,4-naphthaquinone with conc. $H_2SO_4$.

## RESULTS, CALCULATIONS AND DISCUSSION

Table I gives the $R_F$ and $R_M$ values of the compounds required for the various calculations. Two systems were used to obtain data, as certain key compounds migrate too far in one system. Since eqns. (5) and (6) are only obeyed if chromatography is by pure partition, it was essential to eliminate the possibility of adsorption playing a part. Several compounds, therefore, were run in systems A and B, supported on glass paper. They were found to have the same $R_F$ values as on cellulosic paper, and it was assumed that adsorption effects, if they occurred were small enough to be ignored.

In the following calculations, the numerical value of $\Delta R_M$ parameters for groups substituted in an aromatic ring arbitrarily includes a contribution due to the replacement of one hydrogen atom. Thus, $\Delta R_M$(OH) includes the unknown but small subtraction of $\Delta R_M$(aromatic H). This procedure eliminates several tedious repetitions, and no error is introduced into the calculations, as the hydrogen atoms cancel out. The $\Delta R_M$ value for the divalent group (CH=CH—CH=CH) includes the loss of two hydrogen atoms.

*(a) Calculation for thienol*

The $R_F$ value of thienol is (eqns. (5) and (6)) determined by the chromatographic equilibrium of two tautomeric forms. To calculate the position of equilibrium, the $R_F$ values of these two forms must be found. To do this, $\Delta R_M$(OH) is calculated first and then $\Delta R_M$ for the ketonisation of a phenol. Thienol is then converted to a derivative of its pure enol form, which by comparison with a similar derivative of a

TABLE I

$R_F$* AND $R_M$ VALUES OF PHENOLS, *p*-ALKOXYPHENOLS, QUINONES, OXIMES AND *p*-NITROSO COMPOUNDS ON PAPER IMPREGNATED WITH TRIGOL

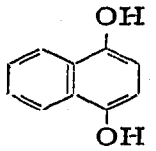
The mobile phase was either (A) di-isopropyl ether or (B) di-*n*-butyl ether

| Compound | System A | | System B | |
|---|---|---|---|---|
| | $R_F$ | $R_M$ | $R_F$ | $R_M$ |
| Phenol | 0.725 | −0.420 | 0.52 | −0.027 |
| *p*-Methoxyphenol | 0.50 | 0.000 | 0.36 | +0.255 |
| 1-Naphthol | 0.76 | −0.509 | 0.64 | −0.252 |
| 2-Naphthol | 0.685 | −0.337 | 0.50 | 0.000 |
| Quinol | 0.10 | +0.954 | 0.02 | +1.644 |
| *p*-Benzoquinone | 0.75 | −0.468 | 0.55 | −0.092 |
| 1,4-Naphthaquinone | 0.86 | −0.802 | 0.80 | −0.592 |
| 2,3-Dihydro-1,4-naphthaquinone | 0.715 | −0.398 | 0.67 | −0.312 |
| 1,4-Naphthaquinol | 0.15 | +0.764 | — | — |
| 2-Phenanthrol | 0.645 | −0.260 | 0.49 | +0.029 |
| 3-Phenanthrol | 0.645 | −0.260 | 0.49 | +0.029 |
| 2-Anthrol | 0.645 | −0.260 | 0.49 | +0.029 |
| 9-Phenanthrol | 0.73 | −0.428 | 0.62 | −0.220 |
| 1-Anthrol | 0.73 | −0.428 | 0.62 | −0.220 |
| *p*-Benzoquinone dioxime | — | — | 0.115 | +0.883 |
| 1,4-Naphthaquinone dioxime | — | — | 0.29 | +0.380 |
| 1,4-Naphthaquinone monoxime | — | — | 0.56 | −0.106 |
| *p*-Nitrosophenol | — | — | 0.20 | +0.602 |
| *p*-Nitrosoanisole | — | — | 0.85 | −0.759 |
| Thienol | 0.71 | −0.397 | | |
| *p*-Benzyloxyphenol | 0.835 | −0.703 | | |
| *p*-(2-Thiophenylmethoxy)-phenol | 0.725 | −0.421 | | |

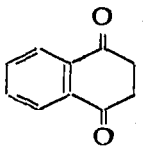
* $R_F$ values were calculated from $R_M$ values and not *vice versa*. (See Part III[15].)

non-tautomeric phenol will yield the hypothetical $R_M$ value of the enol of thienol. $\Delta R_M$ for the ketonisation of a phenol will then give the $R_M$ value of the keto form.

The value for $\Delta R_M$(OH) can be determined by comparing $R_M$(phenol) with $R_M$(quinol). However, it was considered best to obtain the value of this parameter from at least two comparisons as it is of vital significance for the accuracy of the calculation. (In any case, it could not be taken as axiomatic that quinol itself is non-tautomeric, since certain tautomeric properties are observed in other polyhydroxybenzenes.) A further comparison was therefore made with 1,4-naphthaquinol (IX). This substance is readily separable from its tautomeric keto form, 2,3-dihydro-1,4-naphthaquinone (X), and both tautomers are stable under laboratory conditions[5].




(IX)




(X)

However, since in (IX) each OH group is subject to the *ortho*-effect of a *peri*-CH group, the magnitude of this effect must be determined first.

(*i*) $\Delta R_M$(*CH=CH—CH=CH fused to aromatic ring*). This parameter was calcu-

lated (see a previous example[15]) by comparing the $R_M$ values of (a) phenol and 2-naphthol, and (b) 2-naphthol and 2- or 3-phenanthrol. The values were in excellent agreement and

$$\Delta R_M = +0.080 \pm 0.003 \text{ in System A and}$$
$$+0.023 \pm 0.001 \text{ in System B}$$

(*ii*) $\Delta R_M$(*ortho-effect due to peri-CH*). This was calculated by comparing (a) 1- and 2-naphthol, (b) 1- and 2-anthrol, and (c) 2- and 9-phenanthrol. The agreement was excellent.

$$\text{Mean } \Delta R_M = -0.170 \pm 0.002 \text{ in System A and}$$
$$-0.251 \pm 0.002 \text{ in System B}$$

(Comparison of *p*-benzoquinone and 1,4-naphthaquinone using the expression,

$$R_M(p\text{-benzoquinone}) + \Delta R_M(\text{CH=CH—CH=CH}) + 2 \times \Delta R_M(\textit{ortho}\text{-effect}) = R_M(\text{naphthaquinone})$$

gives

$$\Delta R_M(\textit{ortho}\text{-effect}) = -0.207 \text{ in System A and}$$
$$-0.308 \text{ in System B}$$

The *ortho*-effect thus appears to be remarkably constant in this system, even when the functional group varies.)

(*iii*) $\Delta R_M$(OH). Calculation (a) from $R_M$(quinol) and (b) from $R_M$(1,4-naphthaquinol).

$$\text{(a) } \Delta R_M(\text{OH}) = R_M(\text{quinol}) - R_M(\text{phenol}) = +1.451 \text{ in System A}$$

$$\text{(b) } \Delta R_M(\text{OH}) = R_M(\text{1,4-naphthaquinol}) - \Delta R_M(\text{CH=CH—CH=CH}) - R_M(\text{phenol}) - 2 \times \Delta R_M(\textit{ortho}\text{-effect}) = +1.444 \text{ in System A}$$

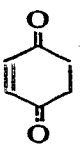
The agreement between the two calculations confirms that there is no tautomerism in quinol and that the $\Delta R_M$ parameters of the two *p*-substituted OH groups in quinol and 1,4-naphthaquinol are additive.

(*iv*) $\Delta R_M$(*ketonisation of OH*). The two key compounds for the calculation of this parameter are the stable tautomers (IX) and (X).

$$\Delta R_M(\text{ketonisation of OH}) = \frac{R_M(\text{X}) - R_M(\text{IX})}{2} = -0.581 \text{ in System A}$$

The value of this parameter in System A can be checked independently by another calculation involving $R_M$(quinol). The latter calculation also yields the parameter in System B, which cannot be found directly as 1,4-naphthaquinol (IX) does not migrate appreciably in the latter system.

Thus, the $R_M$ value for the hypothetical diketo tautomer of quinol (XI) is found from the following expression.



(XI)

$$R_M(\text{XI}) = \Delta R_M(\text{X}) - \Delta R_M(\text{CH=CH—CH=CH}) - 2 \times \Delta R_M(\textit{ortho}\text{-effect})$$
$$= -0.138 \text{ in System A and}$$
$$+ 0.165 \text{ in System B}$$

Then

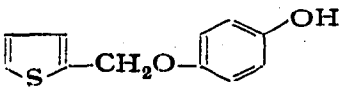
$$\Delta R_M(\text{ketonisation of OH}) = \frac{R_M(\text{XI}) - R_M(\text{quinol})}{2}$$
$$= -0.584 \text{ in System A and}$$
$$-0.739 \text{ in System B}$$

There is complete agreement between the two values for System A.

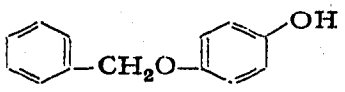
(*v*) $\Delta R_M$(*enol form of thienol*). To calculate the $R_M$ value of the pure enol form of thienol, we need to know $\Delta R_M$(OH), which has already been calculated, and the hypothetical $\Delta R_M$ value for the thiophenyl radical. By the processes of formal summation (described in more detail previously[3]),

$$R_M(\text{enol form of thienol}) = \Delta R_M(\text{thiophenyl radical}) + \Delta R_M(\text{OH})$$

(This summation is legitimate as the constitutive relationships are identical in both halves of the equation.) The value of $\Delta R_M$(thiophenyl radical) can be obtained by chromatographing a suitable 2-thiophenyl compound with an analogous phenyl compound and calculating from their $R_M$ values by the method shown below. We used *p*-(2-thiophenylmethoxy)-phenol (XII), which could be readily prepared from the available 2-chloromethylthiophen, and compared it in System A with *p*-benzyloxyphenol (XIII).




(XII)




(XIII)

Then, the usual formal processes lead to

(a) $R_M(p\text{-benzyloxyphenol}) = \Delta R_M(\text{phenyl}) + \Delta R_M(\text{OH}) + \Delta R_M(\text{CH}_2\text{OPh})$
(b) $R_M(p\text{-thiophenylmethoxyphenol}) = \Delta R_M(\text{thiophenyl}) + \Delta R_M(\text{OH}) + \Delta R_M(\text{CH}_2\text{OPh})$
(c) $R_M(\text{phenol}) = \Delta R_M(\text{phenyl}) + \Delta R_M(\text{OH})$
(d) $R_M(\text{enol form of thienol}) = \Delta R_M(\text{thiophenyl}) + \Delta R_M(\text{OH})$

Therefore,

$$R_M(\text{enol form of thienol}) = R_M(p\text{-thiophenylmethoxyphenol}) - R_M(p\text{-benzyloxyphenol}) + R_M(\text{phenol})$$
$$= -0.138 \text{ in System A}$$

The $R_F$ value of the enol form is thus 0.579.

(*vi*) $R_M$(*keto form of thienol*). This $R_M$ value can be obtained by using the value in (iv) above for $\Delta R_M$(ketonisation).

In System A, therefore,

$$R_M(\text{keto form of thienol}) = -0.138 - 0.581 = -0.719$$

The corresponding $R_F$ value is 0.840.

(*vii*) *The position of equilibrium in thienol* can now be calculated from eqns. (5) and (6), since the $R_F$ values of both forms and the tautomeric mixture are known. In the mobile phase of System A (di-isopropyl ether), the amount of enol form is found to be 40 % and keto form 60 %. In the stationary phase (trigol) there is 70 % enol form and only 30 % keto form. There is thus a higher proportion of keto form in the less polar phase. This is the reverse of what is found in acetoacetic ester and similar keto–enol tautomers. But this can be taken as confirmatory of the theoretical treatment, since the enol form of acetoacetic ester is stabilised by internal hydrogen bonding (which is maximal in non-polar solvents such as hexane and minimal in water because of competitive solvation); in thienol, however, no internal hydrogen bonding is possible and it is to be expected that the enol form would be more soluble in more polar solvents, in agreement with the above findings. Our results agree well with the prediction of HURD AND KREUZ[13], who stated that thienol could be expected to contain roughly equal amounts of keto and enol form.

(*b*) *p-Nitrosophenol*

To determine the tautomeric equilibrium in this compound a similar procedure to that outlined above can be used. First it is necessary to select compounds closely similar to *p*-nitrosophenol but which contain pure non-tautomeric nitroso and oximino groups. By processes of formal addition and subtraction the $R_F$ values of the two tautomeric forms and hence the equilibrium can be calculated. *p*-Nitrosoanisole was selected as a suitable compound containing a non-tautomeric nitroso group.

(*i*) $\Delta R_M(OCH_3)$

$$\Delta R_M(\mathrm{OCH_3}) = R_M(p\text{-methoxyphenol}) - R_M(\mathrm{phenol}) = +\ 0.282 \text{ in System B}$$

(*ii*) *The* $R_M$ *value for the hypothetical nitroso form* (*V*) of *p*-nitrosophenol is found as follows:

$$R_M(\text{nitroso form}) = R_M(p\text{-nitrosoanisole}) - \Delta R_M(\mathrm{OCH_3}) + \Delta R_M(\mathrm{OH}) = +\ 0.630 \text{ in System B}$$

($\Delta R_M$(OH) for System B was calculated from quinol and phenol.)

(*iii*) *The* $R_M$ *value of the hypothetical oxime form* (*VII*) of *p*-nitrosophenol was calculated from *p*-benzoquinone and its dioxime.

$$R_M(\text{oxime form}) = R_M(\text{benzoquinone}) + \frac{R_M(\text{benzoquinone dioxime}) - R_M(\text{benzoquinone})}{2} = +0.396 \text{ in System B}$$

(*iv*) *The* $R_M$ *value of the hypothetical keto-nitroso form* (*VI*) of *p*-nitrosophenol can be calculated as follows:

$$R_M(\text{keto-nitroso form}) = R_M(\text{nitroso form}) + R_M(\text{ketonisation of OH}) = -0.109 \text{ in System B}$$

The experimental $R_M$ value for *p*-nitrosophenol in System B is + 0.602. This is, within experimental error, indistinguishable from the $R_M$ value calculated for the

pure nitroso form (V). There would thus appear not to be more than a few percent of oxime form present. These findings thus support the views of HODGSON[1,2].

*(c) 4-Nitroso-1-naphthol*

The $R_M$ value of the oxime form (1,4-naphthaquinone monoxime) can be calculated, as was the oxime form of *p*-nitrosophenol.

$$R_M(\text{oxime form}) = R_M(\text{naphthaquinone}) + \frac{R_M(\text{naphthaquinone dioxime}) - R_M(\text{naphthaquinone})}{2}$$
$$= -0.592 + 0.486$$
$$= -0.106 \text{ in System B}$$

This is identical with the experimental $R_M$ value for this substance, thus supporting all the other chemical and physical evidence[7] for a 100% oxime structure.

*(d) Ethyl acetoacetate*

It would have been desirable to test the equations described above on a compound whose tautomerism had been quantitatively studied by classical methods. The most promising type of compound appeared to be ethyl acetoacetate, whose tautomeric equilibrium in several solvents[4] has been extensively studied. Attempts were made to chromatograph this substance in two systems, trigol/iso-octane and olive oil/70% (v/v) aqueous ethanol, but only diffuse bands were obtained and $R_F$ values could not be measured. This may have been due to the interconversion of keto and enol forms in ethyl acetoacetate being relatively slow (as it is known to be in the absence of catalysts[4]). Although interconversion is rapid in the presence of an acid or base[4], the addition of either to one phase would introduce a new variable. The enol form of ethyl acetoacetate is known to be stabilised by internal hydrogen bonding. As enolization is affected by pH, the resultant chromatographic effect would be too complex for study.

## SUMMARY

The effect of tautomerism on $R_M$ has been studied. The general nature of the effect on chromatography is discussed and equations obtained by means of which the existence of tautomerism can be verified chromatographically. If sufficient data are available from the study of archetypal compounds in which each form of the tautomeric group occurs free from other constitutive effects, it is possible, by these equations, to calculate the position of tautomeric equilibrium in a given compound. The procedure is illustrated with reference to the tautomerism of thienol (2-hydroxythiophen), *p*-nitrosophenol and 4-nitroso-1-naphthol. The procedure followed rests on the adherence of many group and structural $\Delta R_M$ parameters to the additivity principle of MARTIN's equation.

## REFERENCES

1 H. H. HODGSON, *J. Chem. Soc.*, (1932) 1395.
2 H. H. HODGSON, *J. Chem. Soc.*, (1937) 520.
3 L. C. ANDERSON AND R. L. YANKE, *J. Am. Chem. Soc.*, 56 (1934) 732.
4 C. K. INGOLD, in *Structure and Mechanism in Organic Chemistry*, Bell & Sons Ltd., London, 1953, p. 536.
5 R. H. THOMSON, *Quart. Rev. (London)*, 10 (1956) 27.
6 A. SCHORS, A. KRAAIJEVELD AND E. HAVINGA, *Rec. Trav. Chim.*, 74 (1955) 1243.

[7] *Elsevier's Encyclopaedia of Organic Chemistry*, Vol. 12 B, Elsevier, Amsterdam, 1952, p. 2786.
[8] J. FRANC AND J. JOKL, *Collection Czech. Chem. Commun.*, 24 (1959) 250.
[9] S. MARCINKIEWICZ AND J. GREEN, *J. Chromatog.*, 10 (1963) 372.
[10] R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Biochem. J.*, 38 (1944) 224.
[11] O. DIMROTH, *Ann.*, 377 (1910) 127.
[12] K. H. MEYER, *Ann.*, 380 (1911) 212.
[13] C. D. HURD AND K. L. KREUZ, *J. Am. Chem. Soc.*, 72 (1950) 5543.
[14] J. GREEN AND S. MARCINKIEWICZ, *J. Chromatog.*, 10 (1963) 35.
[15] J. GREEN, S. MARCINKIEWICZ AND D. MCHALE, *J. Chromatog.*, 10 (1963) 158.