TABLE I

Reagent	Product	Labelled amount of oxymetazoline hydrochloride (mg)	mg found		Recovery %
			Proposed method	N.F. method	
Bromo phenol blue	Nasal drop solution (10 ml)	5	5·05	5·1	99·6
Bromo cresol green	Nasal drop solution (10 ml)	5	5·12	5·1	100·2

Assay Procedure

A solution of bromo cresol green or bromo phenol blue (5 ml) was mixed with 5 ml buffer solution (pH 3·4 for bromo cresol green and pH 2·5 for bromo phenol blue) and 3 ml of standard solution. 10 ml of chloroform was added and mixture was shaken for one minute. Sample solution was treated in the same way. Phases were allowed to separate for 15 minutes. Chloroform phase was collected and the absorbance was measured at 420 nm against chloroform blank.

Results

A comparative data of results obtained by proposed as well as N.F.[2] method is reported in Table I.

Discussion

The described method is advantageous because this is the first colorimetric method for the estimation of oxymetazoline hydrochloride. The method can be applied to dilute solutions. Measurement of colour complex is much more convenient than the official titration method. The method is simple and less time consuming as compared to u.v. method. It was observed that bromo cresol green is more sensitive than bromo phenol blue in the estimation of oxymetazoline hydrochloride.

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LCAO-MO METHOD FOR THE EVALUATION OF SPECTRAL SHIFT DUE TO ELECTRON-REPELLING SUBSTITUENTS IN BENZOIC ACID

LCAO-MO method has been utilised for the evaluation of spectral shifts in *p*-band transition of benzoic acid by electron-repelling substituents such as methoxy, chloro and hydroxy groups. In all the cases except in 2-chloro benzoic acid a bathochromic shift is observed. The observed shifts are found to be in agreement with the experimental results.

Introduction

Of all the approximate M.O. theories, LCAO–MO method proved to be a satisfactory and simple device for calculating the spectral shifts observed in the ultraviolet spectrum when an electron repelling group is introduced in organic compounds. D. Peters[1] has successfully applied LCAO–MO theory in alternant hydrocarbons and calculated the effect of methyl-substitution on *p*-, *α*- and *β*-band transition of ultraviolet spectra. Chandra *et al.*[2–3] have calculated the spectral shift in methyl pyridines and phenol with the aid of this theory. A M.O. calculation of ultraviolet absorption spectra of 1 : 5 and 1 : 8 naphthyl pyridine was made by T. E. Peacock[4]. Due to non-availability of experimental data, the $\pi - \pi^*$ transition frequencies in case of organic compounds containing heteromolecules could not be calculated theoretically. In recent years with the help of high precision spectrometers various workers[5–8] have studied the ultra violet spectrum of large number of organic compounds thus making a wide scope for theoretical work. The present method deals with LCAO·MO method for calculating the spectral shift due to some electron-repelling substituents in benzoic acid.

Method

The directing power of substituents in an aromatic ring with regard to further substituent can be very well explained by inductive and hyperconjugative effects. When an electron-repelling substituent replaces a hydrogen atom in an aromatic ring the value of the coulomb integral changes. The net change in transition energy due to inductive effect is given by:

$$(\Delta E_{mn})_{\text{induc.}} = (C_{nr}^2 - C_{mr}^2)\,\delta\alpha_r$$

where C_{mr} and C_{nr} refer to the atomic orbital coefficient at the r-th carbon atom of the m-th highest filled and n-th lowest vacant molecular orbital respectively. $\delta\alpha_r$ is the change in coulomb integral and has

been assigned a value of $-0{\cdot}1\ \beta$ for $-OCH_3$–Cl and –OH substituents.

Coulson-Longuet-Higgins[9] gave an expression for calculating the change in transition energy due to hyperconjugative effect. If β_{rs} refer to the resonance integral of the bond formed between the r-th carbon atom of the benzoic acid and s-th atom of the electron-repelling substituent then Longuet-Higgins and Sowden[10] showed that the energy due to hyperconjugative effect can be written as:

$$(\Delta E_{mn})_{\text{hyperconj.}} = \beta_{rs}^{2}\left(\sum_{k\neq n}\frac{C_{nr}^{2}C_{ks}^{2}}{E_n - E_k} - \sum_{k\neq m}\frac{C_{mr}^{2}C_{ks}^{2}}{E_m - E_k}\right)$$

where C_{ks} refer to the atomic orbital coefficients of the electron-repelling substituent and the summation k refers to both bonding and antibonding molecular orbitals. The values of C_{ks}, E_k and β_{rs} for, $-OCH_3$ –Cl and –OH group are summarized in Table II. The various coulomb and resonance integrals obtained from simple LCAO-MO calculations are summarized in Table I. The numbering refers to Fig. 1 (*a*)[11].

The resultant effect of energy change will be the mere addition of the two effects, thus:

$$(\Delta E_{mn})_{\text{total}} = (\Delta E_{mn})_{\text{induc.}} + (\Delta E_{mn})_{\text{hyperconj.}}$$

$\Delta\lambda$(mu) is the energy change converted into wavelength by taking $\beta = -23{,}000\ cm^{-1}$. The agreement between the calculated and experimental shift is quite consistent as it is evident from Table III. A slight discrepancy in the value of spectral shift is obtained in case of 3-methoxy benzoic acids though the nature of the spectral shift is same. It can be observed from Table III that in the case of methoxy substituted benzoic acid, the transition due to hyperconjugative effect is dominant while in chloro and hydroxy substituents the inductive effect dominates which is qualitatively observed.

TABLE I

Parameters for benzoic acids

Coulomb Integrals a_r	Resonance integrals Hrs. r and s neighbours with ring β
$a_1 - a_c = a_5 - a_c = 0{\cdot}05\ \beta$	$H_{76} \cdots \beta$
$a_2 - a_c = a_4 - a = 0{\cdot}02\ \beta$	$H_{78} \cdots \sqrt{2}\ \beta$
$a_3 - a_c = \cdots = 0\ \beta$	$H_{79} \cdots \sqrt{2}\ \beta$
$a_6 - a_c = \cdots = 0{\cdot}18\ \beta$	All others $\cdots 0$
$a_7 - a_c = \cdots = 0{\cdot}6\ \beta$	Overlapping integrals
$a_8 - a_c = \cdots = 1{\cdot}25\ \beta$	are all taken 0.
$a_9 - a_c = \cdots = 2\beta$	

TABLE II

Group	Energy levels	δa_r	β_{rs}
$-OCH_3$	$a + 2{\cdot}962\ \beta$	$-0{\cdot}1\ \beta$	$0{\cdot}7\ \beta$
	$a + 2{\cdot}751\ \beta$		
	$a - 2{\cdot}024\ \beta$		
–Cl	$a + 2{\cdot}8\ \beta$	$-0{\cdot}1\beta$	$0{\cdot}4\ \beta$
–OH	$a + 2{\cdot}7\ \beta$	$-0{\cdot}1\ \beta$	$0{\cdot}6\ \beta$

TABLE III

Calculated and experimental spectral shift due to electron-repelling substituents in benzoic acid
$(E_n - E_{n'}) = -1{\cdot}5952\ \beta$

Benzoic acid	$(\Delta E_{mn})_{\text{induc}}$	$(\Delta E_{mn})_{\text{hyperconj}}$	$(\Delta E_{mn})_{\text{total}}$	$\Delta\lambda$ (mμ) Calc.	$\Delta\lambda$ (mμ) Expt.[12]
2. methoxy	$-0{\cdot}0063\beta$	$+0{\cdot}0167\beta$	$+0{\cdot}0104\beta$	1·7	2·0
3 methoxy	$+0{\cdot}0069\beta$	$+0{\cdot}0330\beta$	$+0{\cdot}0396\beta$	6·9	2·0
4 methoxy	$+0{\cdot}0123\beta$	$+0{\cdot}1195\beta$	$+0{\cdot}1318\beta$	24·4	21·0
2 : 6 dimethoxy	$-0{\cdot}0126\beta$	$+0{\cdot}0335\beta$	$+0{\cdot}0208\beta$	3·5	4·0
2 chloro	$-0{\cdot}0063\beta$	$+0{\cdot}0000\beta$	$-0{\cdot}0063\beta$	−1·1	1·0
3 chloro	$+0{\cdot}0069\beta$	$+0{\cdot}0069\beta$	$+0{\cdot}0138\beta$	2·3	2·0
4 chloro	$+0{\cdot}0123\beta$	$+0{\cdot}0194\beta$	$+0{\cdot}0317\beta$	5·5	6·0
2 hydroxy	$-0{\cdot}0063\ \beta$	$+0{\cdot}0005\beta$	$-0{\cdot}0058\beta$	−1·0	0·0
3 hydroxy	$+0{\cdot}0069\beta$	$+0{\cdot}0165\beta$	$+0{\cdot}0234\beta$	4·0	4·0
4 hydroxy	$+0{\cdot}0123\beta$	$+0{\cdot}0369\beta$	$+0{\cdot}0592\beta$	9·4	8·0

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ISOLATION OF 6-HYDROXY-2′, 7-DIMETHOXY-4′, 5′-METHYLENEDIOXYISOFLAVONE FROM THE PODS OF *DALBERGIA ASSAMICA*

Dalbergia assamica (family Leguminosae) is a climber having glabrous flexible pods. Seeds of this species are similar to the seeds of *D. latifolia* except that they are more smooth and smaller than the latter. It has been found to contain a rare compound, 6-hydroxy-2′,7-dimethoxy-4′, 5′-methylenedioxyisoflavone, the occurrence of which from *Dalbergia* species is being reported for the first time. This compound has previously been isolated from the heartwood of *Cordyla africana*[1] and *Mildbraedeodendron excelsa*[2]. Air-dried and coarsely powdered pods (1 kg), from which seeds (255 gm) had been removed, were successively extracted with petroleum ether (60–80°), benzene and ethyl acetate. Benzene and ethyl acetate extracts were found to be similar (TLC) and combined while pet. ether extract was worked up separately.

Pet. ether extract on column chromatography over silica gel gave a compound A, while combined benzene and ethyl acetate extracts after column chromatography, preparative TLC and further purification yielded substances A, B, C and D in workable quantities.

Compound A, a white crystalline solid (55 mg) m.p. 140°, $[\alpha]_D$ −28·9° (c, 0·850 in $CHCl_3$); ν_{max}^{KBr}: 3520 (hydroxyl) and 1649 (double bond) cm^{-1}; showed bluish-violet colouration with Liebermann-Burchard reagent (test for sterols); acetate m.p. 127°. It was identified as β-sitosterol and confirmed by comparison (co-TLC, m.m.p.) with an authentic sample. Compound B crystallised from methanol as white needles (80 mg) m.p. 252–53° (*lit*[2] *m.p. 251–52°*); ν_{max}^{KBr}: 3430 (hydroxy), 1640 (carbonyl), 1040 (methoxyl),940 (methylenedioxy)[3], *1618* and *1512* (aryl) cm^{-1}. λ_{max}^{MeOH}: 255, 312 nm, with NaOAc, 255, 312 nm. It gave a positive test for isoflavones and a deep green blue colour on heating with conc. sulphuric acid and gallic acid, indicating the presence of methylenedioxy group[4]. Acetylatiou (Ac_2O-C_5H_5N) afforded a monoacetate as needles from methanol m.p. 215–17°. NMR ($CDCl_3$, δ)[2]: 8·0 (1H, s, C–2), 7·93 (1H, s, C–5), 7·43 (1H, s, C–6′), 7·01 (1H, s, C–3′), 6·75 (1H, s, C–8), 6·0 (2H, s, O–CH_2–O), 4·0, 3·8 (6H, s, 2 OMe), 2·41 (3H, s, $OCOCH_3$). This data indicated the compound to be 6-hydroxy-2′, 7-dimethoxy-4′, 5′-methylenedioxyisoflavone; confirmed by preparation of methyl ether (using K_2CO_3 and dimethyl sulphate in acetone). m.p.[5] 233–34°. MS: m/e 342 (M^+), 311, 167 and 175. Mass spectra of the compound B is also similar to that reported in literature[1].

Compound C crystallised from glacial acetic acid as colourless crystals (20 mg), m.p. 295–96°, ν_{max}^{KBr} 3650 (hydroxyl), 1640 (carbonyl), 955 (methylenedioxy), *1618* and *1508* (aryl) cm^{-1}. λ_{max}^{MeOH}: 249, 295 nm, with NaOAC 257, 296 (inflexion). It gave positive tests both for isoflavones and methylenedioxy grouping and characterised as ψ-baptigenin. Its identity was confirmed by comparison[6,7] (co-TLC, m.m.p. and IR) with an authentic sample.

Compound D, a white crystalline solid (100 mg m.p. 288–90° $[\alpha]_D$ −30·9° (c, 0·934 in $CHCl_3$); ν_{max}^{KBr}: 3470 (hydroxyl) 1660 (double bond) cm^{-1}, showed positive tests for steroids and glycosides. Acid hydrolysis of this compound yielded β-sitosterol and glucose. It was identified as β-sitosterol-β-D-glucoside by comparing IR spectra, co-TLC and m.m.p. determination with an authentic sample.

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