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SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF SOME BROMO-SUBSTITUTED 1,2-BENZISOXAZOLE DERIVATIVES

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ABSTRACT

Several bromo-substituted 1,2-benzisoxazoles have been synthesised by the bromination of 7-acyl (or formyl)-6-hydroxy-3-methyl (or ethyl)-1,2-benzisoxazoles (I, VII) and their corresponding methoxy derivatives (V) under different reaction conditions. The compounds (II) have been cyclised into furano (6,7-*d*)-1,2-benzisoxazoles (XI). The structures of these bromo derivatives have been confirmed by IR, PMR and mass spectral data. All these compounds have also been screened for antitubercular activity.

INTRODUCTION

A NUMBER of 1,2-benzisoxazole derivatives have been reported to possess various types of activities[1–6]. Some amino-1,2-benzisoxazoles have been found to show antitubercular activity[7]. The present paper describes some interesting results obtained in the bromination of 6-hydroxy-7-carbonyl-1,2-benzisoxazoles and their antitubercular activity.

Bromination of I with equimolar bromine in acetic acid at room temperature gave 5-bromo derivatives (II). Formation of these compounds was confirmed by PMR, which showed only one singlet in aromatic region for one deshielded proton (C-4). On the other hand, side chain bromination was observed with corresponding methoxy derivatives (V) under similar reaction conditions. The formation of VI was evidenced from PMR, which showed two doublets in the aromatic region for two *ortho* protons (J = 9 Hz) and downfield singlet for $-COCH_2Br$ in case of VI*a*, *b* and a quartet for $-CO.CH(Br)CH_3$ in case of VI*c* and a prominent M^+-Br peak in the mass spectrum. Similarly bromination of VII at room temperature gave 5-bromo derivatives (VIII) as revealed by their PMR spectral data (Scheme 1).

Dibromo derivatives (III) were obtained by the bromination of I using 2 moles of bromine in acetic acid at water-bath temperature. The formation of these dibromo compounds (III) was confirmed by PMR spectral data and M^+ ions. Compounds III were also obtained by the bromination (1 mole Br_2) of II under the same reaction conditions and characterized by mixed m.p.

The *o*-hydroxy-acyl compounds (II) were converted into furano (6,7-*d*)-1,2-benzisoxazoles (XI) by Kostanecki and Tambor method[8] (Scheme 2). The formation of XI and intermediate compounds (IX and X) were confirmed by IR and PMR (see experimental).

Antitubercular Activity

Almost all compounds were tested for their antitubercular activity against "*Mycobacterium tuberculosis*" ($H_{37}RV$) by Doub and Youman's method[9]. The minimum concentration of the compounds (in (μg/ml) which completely inhibited the growth of the test organism is recorded (*see* Table I). For comparison the activity under similar conditions of streptomycin is 1 μg/ml and that of INH is 0·05 μg/ml. Compounds II*a*, II*b*, III*a*, III*b* and IX*c* have shown maximum activity.

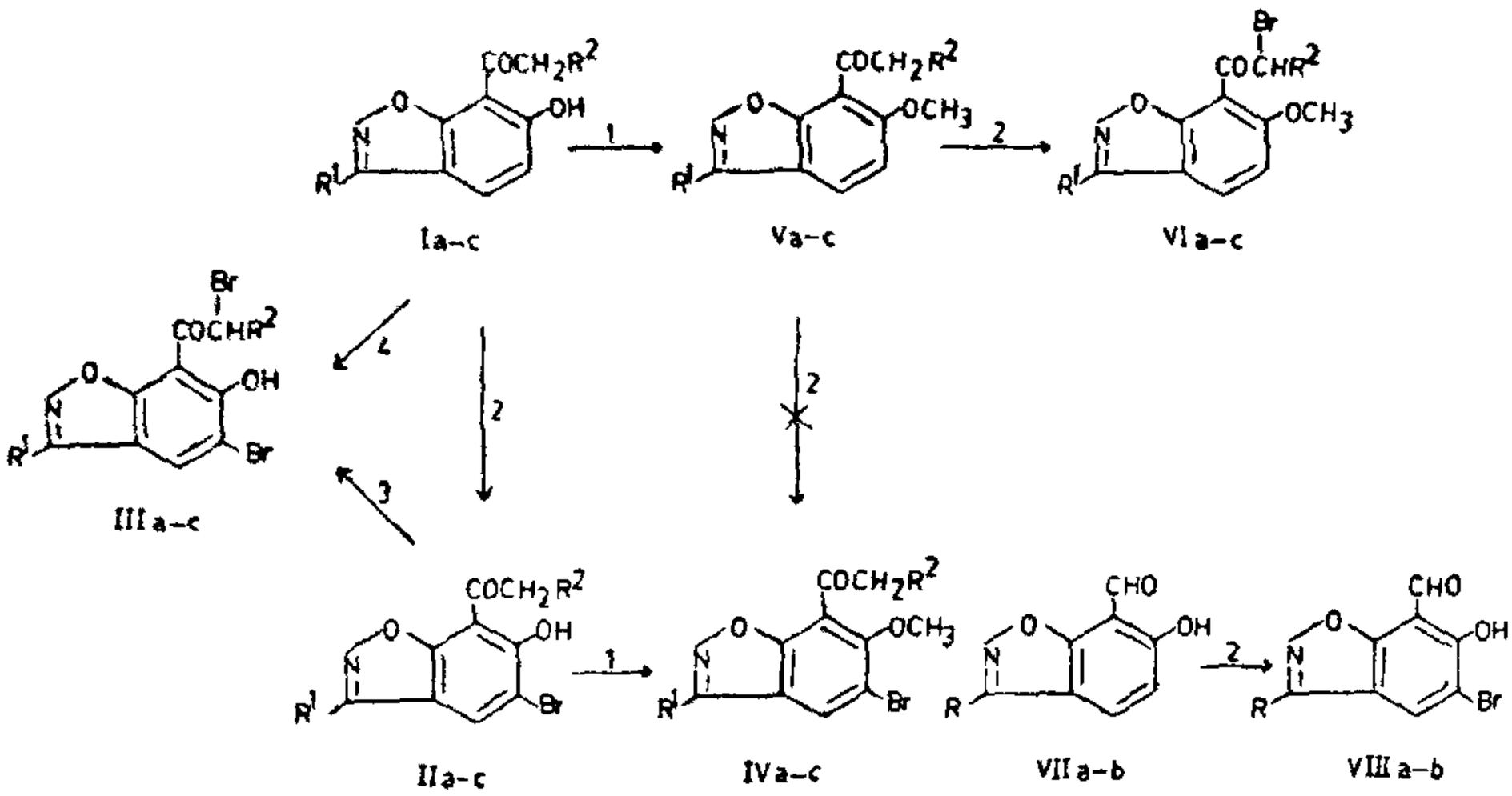
EXPERIMENTAL PROCEDURE

All the melting points are uncorrected. All compounds gave satisfactory C, H and N analysis. IR spectra (ν_{max} in cm^{-1}) were taken in nujol on Perkin-Elmer Infracord 137B spectrometer. PMR spectra were recorded on Varian T–60 instrument with TMS as internal reference and chemical shifts are expressed in δ scales. Mass spectra (MS) were recorded on a CEC–21–110B mass spectrophotometer.

The starting compounds, 6-hydroxy-7-acyl (or formyl) 3-methyl (or ethyl)-1,2-benzisoxazoles (I*a-c* and VII*a*,*b*) were prepared as reported earlier[10–11].

7-*Acetyl*-6-*methoxy*-3-*methyl*-1,2-*benzisoxazole* (V*a*)

An acetone solution of 7-acetyl-6-hydroxy-3-methyl-1,2-benzisoxazole (I*a*, 3 g) was refluxed with K_2CO_3 (5 g) and dimethyl sulphate (2·8 g) for 6 hr. The solvent was removed on a water-bath and water (100 ml) was added to the residue. The solid so obtained was filtered, washed with dilute solution of NaOH, dried and crystallized from ethanol to give 2·4 g (75%) of V*a*, m.p. 131°. IR : 1655, 1595, 825,



SCHEME 1

PMR (CCl_4): 2·26 (*s*, 3H, $-CH_3$), 2·73 (*s*, 3H, $-COCH_3$), 4·06 (*s*, 3H, $-OCH_3$), 7·06 (*d*, J = 9 Hz, 1H, Ar–H at C–5) and 7·43 (*d*, J = 9 Hz, 1H, Ar–H at C–4).

Similarly other methlxy compounds (V*b* and V*c*) were prepared.

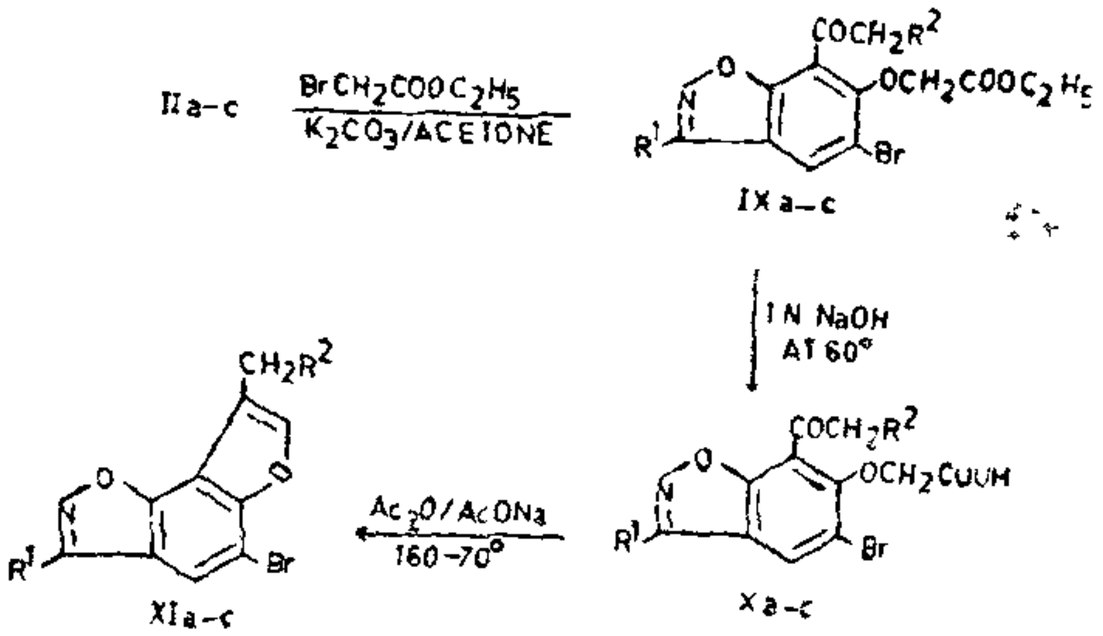
7-Acetyl-5-bromo-6-hydroxy-3-methyl-1, 2-*benzisoxazole* (*IIa*)

To a solution of 7-acetyl-6-hydroxy-3-methyl-1,2-benzisoxazole (I*a*, 1·91 g) in acetic acid (10 ml) a solution of bromine (1·6 g) in acetic acid (10 ml) was added and kept at room temperature for 2 hr. It was then poured over ice-water. The solid so obtained was filtered, washed with water, dried and crystallized from 80% ethanol to give 2·4 g (88%) of II*a*, m.p. 124–5°; IR : 1640, 1590, 830; PMR (CCl_4) : 2·53 (*s*, 3H, $-CH_3$), 2·87 (*s*, 3H, $-COCH_3$), 7·63 (*s*, 1H, Ar–H at C–4) and 14·00 (*s*, 1H, –OH), MS : 269/271 (M^+).

Other monobromo compounds (II*b*, II*c*, IV*a–c*, VI*a–c*, VIII*a* and VIII*b*) prepared by the above procedure are listed in Table I.

5-Bromo-7 (ω-bromoacetyl)-6-hydroxy-3-methyl-1,2-*benzisoxazole*

(III*a*) : A mixture of 7-acetyl-6-hydroxy-3-methyl-1,2-benzisoxazole (I*a*, 1·91 g), bromine (3·2 g) and



SCHEME 2

acetic acid (30 ml) was heated on a water-bath for 3 hr. Working up in usual manner gave dibromo compound, III*a* (3 g, 85%), which was crystallized from acetic acid, m.p. 195°. IR : 1640, 1585, 800; PMR (TFA): 2·70 (*s*, 3H, $-CH_3$), 4·83 (*s*, 2H, $-CH_2$) and 8·15 (*s*, 1H, Ar–H at C–4), MS : 347/349/351 (M^+).

III*b* and III*c* were also prepared by following the above procedure. Their physical data is given in Table I.

TABLE I

Physical, spectral data and antitubercular activity of 1,2-*benzisoxazole derivatives*

Comp. No.	m.p. °C	Yield %	Antitubercular activity in μg/ml	IR cm^{-1}	MS** M^+
1	2	3	4	5	6
IIa*	..	..	5	..	..
IIb	112–4[e]	85	2	1635, 1600, 820	283/285
IIc	146–7[d]	87	Inactive	1630, 1590, 825	..
IIIa*	..	..	2	..	..
IIIb	154–5[k]	80	2	1650, 1600, 800	361/363/365
IIIc	138–9[d]	80	..	1630, 1600, 805	361/363/365
IVa	119[h]	70	Inactive	1700, 1600, 845	283/285
IVb	b.p. 135–40/ 0·4 mm	65	..	1700, 1590, 840	..
IVc	74–5[h]	75	50	1700, 1600, 810	297/299
Va*	..	..	Inactive	..	..
Vb	94[g]	65	Inactive	1660, 1600, 825	..
Vc	101[e]	70	100	1670, 1600, 820	..
VIa	152–3[j]	85	Inactive	1680, 1605, 820	283/285
VIb	118[a]	80	Inactive	1670, 1600, 825	297/299
VIc	104[b]	85	Inactive	1660, 1600, 810	297/299
VIIIa	131–2[d]	80	25	1650, 1595, 760	255/257
VIIIb	93–4[e]	80	25	1655, 1600, 775	..
IXa*	..	..	100	..	..
IXb	57–8[g]	65	50	1745, 1700, 1600	..
IXc	81–2[f]	60	3·12	1760, 1690, 1585	..
Xb	145–7[f]	75	..	1740, 1700, 1600	..
Xc	151–3[i]	75	..	1740, 1700, 1600	..
XIb	90[f]	65	..	1620, 1590, 1075, 850	..
XIc	156–7[d]	75	..	1630, 1595, 1070, 845	..

* The physical and spectral data of these compounds are given in experimental.

** m/e values refer to the isotopic cluster at M^+.

d = Crystallized from ethanol; *e* = crystallized from 80% ethanol; *f* = crystallized from 70% ethanol; *g* = crystallized from 60% ethanol; *h* = crystallized from 50% ethanol; *i* = crystallized from 40% ethanol; *j* = crystallized from acetic acid; *k* = crystallised from 80% acetic acid.

7-Acetyl-5-bromo-6(carbethoxymethoxy)-3-methyl-1,2-benzisoxazole (IXa)

A mixture of the compound IIa (2·7 g, 0·01 mole), anhyd. K_2CO_3 (2·8 g, 0·02 mole), bromoacetic ester (1·67 g, 0·01 mole) and acetone (50 ml) was refluxed for 10 hr. Acetone was distilled off and residue was treated with ice-water. Usual work-up followed by crystallisation from 70% ethanol gave 2·13 g (60%) of IXa, m.p. 70°, IR: 1735, 1690, 1590; PMR (CCl_4): 1·33 (*t*, J = 7 Hz, 3H, $-CH_2CH_3$), 2·50 (*s*, 3H, $-CH_3$), 2·70 (*s*, 3H, $-COCH_3$), 4·23 (*q*, J = 7 Hz, 2H, $-CH_2CH_3$), 4·56 (*s*, 3H, $-OCH_3$) and 7·80 (*s*, 1H, Ar–H at C–4).

Similarly other carbethoxy methoxy compounds (IXb and IXc) were prepared.

7-Acetyl-5-bromo-6-(carboxymethoxy)-3-methyl-1,2-benzisoxazole (Xa)

The ester (IXa, 1·8 g, 0·005 mole) and 1N sodium hydroxide (10 ml) was warmed on a water-bath for

30 minutes. The cold clear solution on neutralisation with dil. HCl gave 1·12 g (70%) of X*a*. It was crystallized from 40% ethanol, m.p. 230–31° (d). IR : 1730, 1690, 1585.

Similarly prepared were X*b* and *Xc*.

3,3'-Dimethylfurano (6,7-*d*)-1,2-*benzisoxazole* (*XIa*)

A mixture of phenoxy acetic acid (X*a*, 0·8 g), acetic anhydride (8 ml) and fused sodium acetate (1 g) was heated on an oil-bath at 160–170° for about 4 hr. The cold reaction mixture was poured over ice-water and worked up as usual to give 0·470 g (70%) of XI*a*. It was crystallized from ethanol, m.p. 139–40°; IR : 1625, 1570, 1075, 845; PMR (CCl_4) : 2·60 (*s*, 6H, 2×CH_3) and 7·53 (*s*, 2H, Ar–H at C–4 and C–2').

XI*b* and XI*c* were prepared as discussed above.

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VARIATIONS IN THE CELLULAR CONSTITUENTS OF *ASPERGILLUS NIDULANS* GROWN ON DIFFERENT CARBON SOURCES

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ABSTRACT

Aspergillus nidulans was grown on six different sugars—glucose, fructose, sucrose, maltose, lactose and galactose; maximum growth was observed in glucose and fructose while lactose and galactose were poor carbon sources. Growth on lactose and galactose revealed marked variations in the levels of various cellular constituents when compared to growth on other sugars. Growth on lactose, particularly, showed significant decrease in the total carbohydrate, glycogen and lipid content, and pronounced increase in nucleic acids (DNA and RNA) levels; growth in this sugar also implied cell shrinkage.

INTRODUCTION

THE utilization of sugars by *Aspergillus nidulans* has been studied in detail by Agnihotri[1–4], Mehrotra and Agnihotri[5,6] and Roberts[7]. Similar studies have also been conducted in other microorganisms such as the *Candida* group[8], *Clostridium tetani*[9], *Mycoplasma agalactiae*[10], *Aspergillus niger*[11] and *Penicillium digitatum*[12]. However, no report has so far appeared regarding the changes observed in the cellular constituents of *A. nidulans* grown with different carbon sources in the medium. The results of such study are presented in this paper.

MATERIALS AND METHODS

Organism : A wild strain of *A. nidulans* with green conidia (Glasgow stock of strain) was used