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SYNTHESIS OF PYRAZOFURINS: FURTHER CORRELATIONS BETWEEN CONFIGURATION–CONFORMATION AND ^{1}H-N.M.R. DATA FOR *C*-(2,3-*O*-ISOPROPYLIDENEGLYCOFURANOSYL) DERIVATIVES

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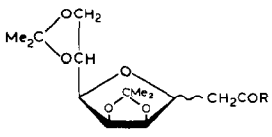
ABSTRACT

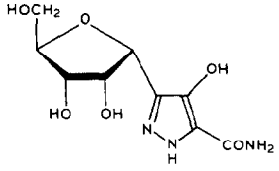
Methyl 4-(2,3-*O*-isopropylidene-5-*O*-trityl-α- and -β-D-ribofuranosyl)-3-oxobutanoate (**5α** and **5β**) were prepared in good yield by reacting 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranose with 3-methoxycarbonylacetonylidenetriphenylphosphorane catalysed by benzoic acid in dry benzene. The mixture of β-ketoesters **5αβ** was transformed into pyrazofurin and pyrazofurin B. The anomeric configuration of these and other *C*-glycoside analogues can be assigned on the basis of ^{1}H-n.m.r. data.

INTRODUCTION

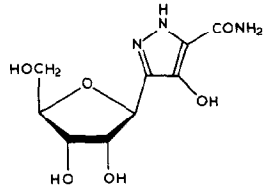
We have reported[1] the synthesis of the *manno* analogues of pyrazofurin, namely, 4-hydroxy-3-(α- and -β-D-mannofuranosyl)pyrazole-5-carboxamides, from



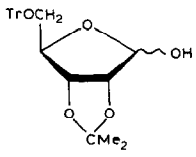
1αβ R = CH_2CO_2Me



2α



2β



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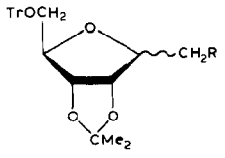
$Ph_3P{=}CHCOCH_2CO_2Me$

4



5αβ R = $COCH_2CO_2Me$
6αβ R = $C(OH)CHCO_2Me$
7αβ R = $COMe$
13αβ R = $COCN_2CO_2Me$

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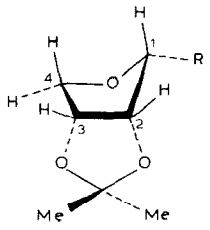
methyl 4-(2,3:5,6-di-*O*-isopropylidene-α- and -β-D-mannofuranosyl)-3-oxobutanoate (**1α** and **1β**), the Wittig reaction products of 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose with 3-methoxycarbonylacetonylidenetriphenylphosphorane.

Because of the biological interest[2] of the pyrazofurins (**2α** and **2β**), four different syntheses have been reported[3] and we now describe a new synthesis from the readily accessible methyl 4-(2,3-*O*-isopropylidene-5-*O*-trityl-α- and -β-D-ribofuranosyl)-3-oxobutanoates (**5α** and **5β**).

The anomeric configurations of some 2,3-*O*-isopropylidene-D-ribofuranosyl *C*-glycosides and *C*-nucleosides have been assigned on the basis of ^{13}C-n.m.r. data[4]. Because of the similar values of $J_{1,2}$ for the α- and β-D-*ribo* anomers, ^{1}H-n.m.r. spectroscopy is not very informative[5]. However, a study of previously reported data[4,6] suggests a new and general ^{1}H-n.m.r. method for assigning these configurations, which may be applied to *C*-(2,3-*O*-isopropylideneglycofuranosyl) derivatives.

RESULTS AND DISCUSSION

The highest yield and the shortest reaction time for the reaction between 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranose (**3**) and 3-methoxycarbonylacetonylidenetriphenylphosphorane (**4**) was achieved by using dry benzene and a catalytic amount of benzoic acid (50-h reflux). An almost quantitative yield of the mixture **5αβ** accompanied by the enols **6αβ** was obtained. Because their separation is difficult, the mixture of **5αβ** and **6αβ** was employed for subsequent transformations. The presence of traces of water in the reaction medium may produce a



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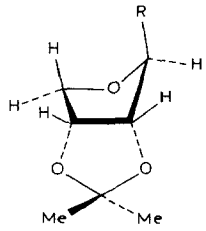

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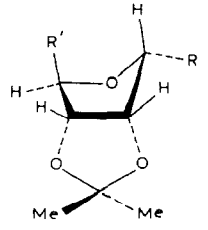

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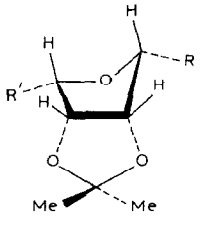

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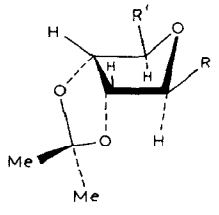

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TABLE I

COUPLING CONSTANTS (Hz) FOR COMPOUNDS OF TYPES **8** AND **11**

R	*R'*	*Type*	*Compound*	$J_{1,2}$	$J_{2,3}$	$J_{3,4endo}$	$J_{3,4exo}$	*Ref.*
-CHO	—	**8**	**16**	4.0	5.2	0.0	3.0	9
-CH=CH-CO_2Me	—	**8**	**17a**	3.0	—	0.0	3.0	9
-CH=CH-CO_2Et	—	**8**	**17b**	—	—	0.0	3.4	9
-$COCH_2CO_2$Me	—	**8**	**18**	4	7	0.0	4.0	9
-CH(OH)CH_2CO_2Me	—	**8**	**19**	2.8	6.3	0.0	2.8	9
4-Hydroxy-2-methylthiopyrimidin-6-yl	—	**8**	**20**	3.5	—	0.0	3.5	9
4-Hydroxy-2-mercaptopyrimidin-6-yl	—	**8**	**21**	3.0	—	0.0	3	9
2,2-Dimethyl-1,3-dioxolan-4-yl	-$CH(CO_2Et)_2$	**11**	**22**	3	6	—	3.5	4

TABLE II

COUPLING CONSTANTS (Hz) FOR COMPOUNDS OF TYPE **9**

R	*Compound*	$J_{1,2}$	$J_{2,3}$	$J_{3,4endo}$	$J_{3,4exo}$	*Ref.*
-CH=N-NH-Ar	**23**	≃1	6.0	<1	3.6	10
-C(Br)=N-NH-Ar	**24**	<0.5	5.9	<0.5	≃3.5	10
-C(C≡CH)=N-NH-Ar	**25**	≃1	6.2	—	—	10
-C(C≡C-Ph)=N-NH-Ar	**26**	≃1	6.1	1.5	3	10
1-(*p*-NO_2-Phenyl)pyrazol-3-yl	**27**	<0.5	6.4	<0.5	4	10
1-(*p*-NO_2-Phenyl)-5-phenylpyrazol-3-yl	**28**	<0.5	6.4	<0.5	3.6	10
4,5-Di(methoxycarbonyl)-1-(*p*-NO_2-phenyl)pyrazol-3-yl	**29**	<0.5	6.3	1.6	3.3	10
[a]	**30**	<0.5	6.0	<0.5	3.5	10
3,4-Di(methoxycarbonyl)pyrazol-5-yl	**31**	1.2	—	—	—	11
3-Carboxamido-4-methoxycarbonylpyrazol-5-yl	**32**	0	—	—	—	11
-CH=N^+(O$^-$)-Me	**33**	0	6.5	0	3.75	12

[a] Dihydro-1,4-di-(2,3-*O*-isopropylidene-β-D-erythrofuranosyl)-3,6-di-(*p*-nitrophenyl)-1,4-tetrazine-1,2,4,5[10].

TABLE III

COUPLING CONSTANTS (Hz) FOR COMPOUNDS OF TYPE **10**

R	*R'*	*Compound*	$J_{1,2}$	$J_{2,3}$	$J_{3,4endo}$	*Ref.*
$-CH_2COCH_2CO_2Me$	CH_2OTr	**5α**	4	6	0.8	[a]
$-CH_2C(OH)=CHCO_2Me$	CH_2OTr	**6α**	4	6	1.0	[a]
$-CH_2COC(N_2)CO_2Me$	CH_2OTr	**13α**	—	—	0.0	[a]
4-Hydroxy-3-methoxycarbonylpyrazol-5-yl	CH_2OTr	**14α**	4	—	1.0	[a]
3-Carboxamido-4-hydroxypyrazol-5-yl	CH_2OTr	**15α**	3.5	—	—	[a]
$-C\equiv C-CO_2Et$	CH_2OTr	**34**	4.7	6.0	≃0	6b
$-C(NH_2)=CHCO_2Et$	CH_2OTr	**35**	4.6	5.8	≃0	6b
5-Ethoxycarbonyl-2-hydroxy-4-mercaptopyrimidin-6-yl	CH_2OTr	**36**	4.6	5.8	≃0	6b
$-CH(N<)=CHCO_2Et$	CH_2OTr	**37**	4.4	6.1	≃0	6b
$-COCH_2CO_2Et$	CH_2OTr	**38**	4.9	6.1	≃0	6b
4-Hydroxy-2-mercaptopyrimidin-6-yl	CH_2OTr	**39**	4.6	6.0	≃0	6b
s-Triazolo[4.3-*a*]pyridin-3-yl	CH_2OH	**40**	4.2	6.3	0.7	6b
$-CH(CO_2Me)_2$	CH_2OTr	**41**	3.5	6	0	4
$-CH(CO_2Et)_2$	CH_2OTr	**42**	3.5	6	0	4
$-CH_2CO_2Me$	CH_2OTr	**43**	4	6	0	4
$-CH_2CN$	CH_2OTr	**44**	3.5	6	0	4
$-CH_2CO_2Me$	CH_2OH	**45**	4	6	0	4
$-CH_2CN$	CH_2OH	**46**	4	6	0	4
-2,2-Dimethyl-1,3-dioxolan-4-yl	$-CH_2CO_2Et$	**47**	3.5	—	≃0	4
-2,2-Dimethyl-1,3-dioxolan-4-yl	$-CH_2CO_2Me$	**48**	3.5	6	≃0	4
-2,2-Dimethyl-1,3-dioxolan-4-yl	$-CH_2COCH_2CO_2Me$	**49**	4	6	≃0	1
-2,2-Dimethyl-1,3-dioxolan-4-yl	$-CH_2COCN_2CO_2Me$	**50**	4	6	≃0	1
-2,2-Dimethyl-1,3-dioxolan-4-yl	4-Hydroxy-3-methoxycarbonyl-5-yl	**51**	3	5	≃0	1
-2,2-Dimethyl-1,3-dioxolan-4-yl	3-Carboxamido-4-hydroxypyrazol-5-yl	**52**	4	6	≃0	1

[a] This paper.

TABLE IV

COUPLING CONSTANTS (Hz) FOR COMPOUNDS OF TYPE **12**

R	*R'*	*Compound*	$J_{1,2}$	$J_{2,3}$	$J_{3,4endo}$	*Ref.*
$-CH_2COCH_2CO_2Me$	CH_2OTr	**5β**	5	4.5	4	[a]
$-CH_2C(OH)=CHCO_2Me$	CH_2OTr	**6β**	4.5	6.5	—	[a]
$-CH_2COC(N_2)CO_2Me$	CH_2OTr	**13β**	—	—	4.5	[a]
4-Hydroxy-3-methoxycarbonylpyrazol-5-yl	CH_2OTr	**14β**	4	6	4	[a]
3-Carboxamido-4-hydroxypyrazol-5-yl	CH_2OTr	**15β**	4	6	3.5	[a]
$-C\equiv C-CO_2Et$	CH_2OTr	**53**	2.8	5.8	1.8	6b
3-Amino-4-hydroxypyrimidin-6-yl	CH_2OTr	**54**	3.0	6.4	4.3	6b
$-C\equiv C-CONH_2$	CH_2OTr	**55**	2.8	5.8	1.5	6b
$-C\equiv C-CO_2Me$	CH_2OTr	**56**	2.9	5.8	1.5	6b
	CH_2OTr	**57**	4.5	—	—	6b
$-COCH_2CO_2Et$	CH_2OTr	**58**	4.1	6.4	—	6b
4-Hydroxy-2-mercaptopyrimidin-6-yl	CH_2OTr	**59**	3.6	5.0	4.5	6b
$-CH(CO_2Me)_2$	CH_2OTr	**60**	4	6.5	4.5	4
$-CH(CO_2Et)_2$	CH_2OTr	**61**	3.5	6.5	5	4
$-CH_2CO_2Me$	CH_2OTr	**62**	4	6	3.5	4
$-CH_2CN$	CH_2OTr	**63**	4.5	6.5	3.5	4
4-Carboxamidothiazol-2-yl	CH_2OH	**64**	4	6	3	13
$-CH_2CO_2Me$	CH_2OH	**65**	4.5	7	4	4
$-CH_2CN$	CH_2OH	**66**	5	6.5	3.5	4

[a] This paper.

TABLE V

AVERAGE COUPLING CONSTANTS (Hz) FOR COMPOUNDS OF TYPES **8–12**

Type	$J_{1endo,2}$	$J_{1exo,2}$	$J_{3,4endo}$	$J_{3,4exo}$	*Conformation*
8	—	2.8–4	0	2.8–4	E_o
9	<1.2	—	<1.6	3.3–3.75	E_o
10	—	3–4.9	<1	—	E_o
11	—	3	—	3.5	E_o
12	2.8–5	—	1.5–4.5	—	^oE

secondary product, 1-(2,3-*O*-isopropylidene-5-*O*-trityl-α- and -β-D-ribofuranosyl)-propanone (**7αβ**), as reported[1] for a similar reaction.

Some assignments of anomeric configuration of *C*-(2,3-*O*-isopropylidene-glycofuranosyl) derivatives have been based on polarimetry[7] and ^{13}C-[4] and ^1H-n.m.r. data[5]. The anomeric configuration of the components of the mixture **5αβ** + **6αβ** could not be effected on the basis of the chemical shift differences of the two singlets for each 2,3-*O*-isopropylidene group[5a] or the $J_{1,2}$ values (4–5 Hz). However, as Moffatt and co-workers[4] indicated, the α-D-ribofuranosyl derivatives can be characterised by their zero $J_{3,4}$ values. MacCoss *et al.*[8] obtained similar results for several α-D-ribonucleosides.

The diagnostic value of $J_{3,4}$ noted for D-ribofuranosyl derivatives may be extended to other 2,3-*O*-isopropylidene-D- or -L-glycofuranosyl derivatives, particularly in conjunction with the $J_{1,2}$ values. The $J_{1,2}$ and $J_{3,4}$ values of some 2,3-*O*-isopropylidene *C*-glycosyl derivatives shown in Tables I–IV suggest a more direct method for making these assignments, which would eliminate the need for comparative analysis of the pairs of anomers. Thus, any *C*-(2,3-*O*-isopropylidene-α- or -β-, -D- or -L-glycofuranosyl) derivative would have one of the general structures **8–12** or their mirror images; **5α** and **5β** are examples of structures **10** and **12**, respectively, and **1α** and **1β** are examples of structures **10** and **11**, respectively. However, H-1,2,3,4 of **10** and **11** correspond now to protons H-4,3,2,1 of **1α** and **1β**, respectively. Further, the 2,3-*O*-isopropylidene group should affect the conformational equilibrium of the tetrahydrofuran ring. Thus, the ^oE or E_o conformations become important in this type of equilibrium. The greater stability of the E_o conformation in type **8** compounds (R=H) may be explained by the 1,2-*cis* and 1,3-*cis* interactions in type **8** and **12** structures (R=R′=H).

This type of E_o conformation has been reported for **41–46** in Table III. In addition, for **44** (type **10**, Table III), a lack of change in the ^1H-n.m.r. spectra in the temperature range from −50° to +70° has been reported[6a], which shows that, even in solution, the conformation is markedly rigid. This effect has been attributed to the tendency of the bulky *endo*-substituent of the type **10** structure to assume a quasi-equatorial orientation, which is compatible only with the E_o conformation. A similar situation might occur in compounds of types **8** and **11**, which also have *endo*-substituents (Table I).

TABLE VI

CHEMICAL SHIFTS[a] (δ) FOR **5**, **6**, AND **13–15**

Solvent		*H-1'*	*H-2'*	*H-3'*	*H-4'*	*H-5'a*	*H-5'b*	*H-2a*	*H-2b*	*H-4a*	*H-4b*	*CMe_2*	*Others*
5α	$CDCl_3$	4.47 ddd	4.68 dd	4.54 dd	4.05 m	3.11 dd	2.99 dd	3.41 d	3.44 d	2.79 dd	2.87 dd	1.38 1.20	3.61 s (OMe) 7.4–7.0 m (Ar)
5β	$CDCl_3$	4.18 ddd	4.49 dd	4.34 dd	4.0 m	3.15 dd	3.04 dd	3.36 d[b]	3.39 d[b]	2.70 dd	2.79 dd	1.45 1.22	3.57 s (OMe) 7.4–7.0 m (Ar)
6α	$CDCl_3$	4.7–4.6 m[b]	4.62 dd	4.59 dd	4.1–4 m[b]	3.15 dd	2.98 dd	—	—	3.36 d	3.39 d	1.39 1.21	3.62 s (OMe) 7.4–7.0 m (Ar) 5.09 s (CH=)
6β	$CDCl_3$	4.5–4.4 m[b]	4.65 m[b]	4.38 dd	4.02 m[b]	3.16 dd	3.02 dd	—	—	3.36 d	3.39 d	1.38 1.20	3.60 s (OMe) 7.4–7.0 m (Ar) 5.02 s (CH=)
13α	$CDCl_3$	5.04–4.5 m			4.18 t	3.4–3.05 m		—	—	3.4–3.05 m		1.45 1.28	3.75 s (OMe) 7.5–7.1 m (Ar)
13β	$CDCl_3$	4.8–4.3 m			4.15 dt	3.0–3.5 m		—	—	3.0–3.5 m		1.50 1.30	3.70 s (OMe) 7.6–7.1 m (Ar)
14α	$CDCl_3$	5.35 d	5.1–4.7 m		4.25 m	3.5–3.1 m		—	—	—	—	1.50 1.30	3.8 s (OMe) 7.5–7.35 m (Ar)
14β	$(CD_3)_2CO$	5.06 d	5.18 d	4.70 dd	4.25 dt	3.17 d		—	—	—	—	1.50 1.30	3.85 s (OMe) 7.5–7.0 m (Ar)
15α	$(CD_3)_2CO$	5.40 d	5.0–4.7 m		4.28 t	3.3 d		—	—	—	—	1.51 1.31	7.4–7.1 m (Ar)
15β	$(CD_3)_2CO$	5.08 d	5.21 dd	4.7 dd	4.25 dt	3.14 d		—	—	—	—	1.51 1.31	7.4–7.1 m (Ar)

[a]At 60 MHz except for **5** and **6** (250 MHz). [b]Unresolved.

TABLE VII

COUPLING CONSTANTS (Hz) FOR **5, 6,** AND **13–15**

	$J_{2a,2b}$	$J_{4a,4b}$	$J_{4a,1'}$	$J_{4b,1'}$	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{5'a,5'b}$
5α	13	17	6.5	6.5	4	6	0.8	3.5	4.5	10
5β	12	16	7.5	5.5	5	6.5	4.5	4	4.5	10
6α	—	[a]	4.5		4	6	1	3.5	4.5	10
6β	—	[a]	[a]		4.5	6.5	[a]	4	4.5	10
13α	—	[a]	[a]		[a]	[a]	0	[a]		[a]
13β	—	[a]	[a]		[a]	[a]	4.5	4.5	[a]	[a]
14α	—	—	—	—	4	[a]	1	[a]	[a]	[a]
14β	—	—	—	—	4	6	4	6		[a]
15α	—	—	—	—	3.5	[a]	[a]	5		[a]
15β	—	—	—	—	4	6	3.5	5		[a]

[a]Unresolved.

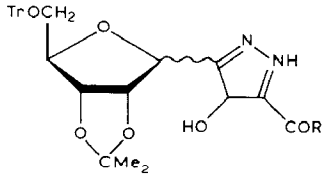
All compounds in this series should show similar $J_{H,H}$ values and a set of coupling constants can be used to establish a particular conformation or structure. Tables I–IV include various compounds of types **8–12**. The average coupling constants for the five types **8–12** given in Table V support this argument.

It may be noted that (*a*) the coupling constants of H-1*exo* and/or H-4*exo* with vicinal *cis* protons are similar in the E_0 conformation (types **8–11**), with $J_{1exo,2}$ and $J_{3,4exo}$ values between 3 and 5 Hz; (*b*) the coupling constants for H-1*endo* and/or H-4*endo* with vicinal *trans* protons reflect the ^0E or E_0 conformation; thus, values of $J_{1endo,2}$ or $J_{3,4endo}$ between 2.8 and 5 Hz point to stronger ^0E character with quasi-axial orientation of the corresponding *endo*-protons, whereas values of ~1.5 Hz, point to stronger E_0 character with quasi-equatorial orientation of the *endo*-protons; (*c*) these different sets of values for $J_{1,2}$ and $J_{3,4}$ for the structures **8–12** allow characterisation of these *C*-glycosyl derivatives, including their anomeric configuration; (*d*) the type **12** structure will acquire appreciable E_0 character when the nature of substituents leads to low 1,3-*cis* R/R′ interactions. In such compounds, $J_{1endo,2}$ and $J_{3,4endo}$ will have reduced values as illustrated by **53, 55,** and **56** in Table IV, where R′ is an acetylenic substituent (-C≡C-CO_2Et, -C≡C-$CONH_2$, and -C≡C-CO_2Me). Structure **9** is a characteristic example of this situation.

The application of the foregoing guidelines to **5α** and **5β** indicates their structures (α anomer, $J_{1,2}$ 4, $J_{3,4}$ 0.8 Hz; β anomer, $J_{1,2}$ 5, $J_{3,4}$ 4.5 Hz). In these examples, the most valuable datum is $J_{3,4}$, instead of $J_{1,2}$ used in other situations. In like manner the anomeric configurations of **6α** and **6β** are indicated.

The mixture of **5αβ** and **6αβ** was treated with equimolecular amounts of tosyl azide and triethylamine in acetonitrile at room temperature, to give an almost quantitative yield of a 3:4 mixture of the diazo derivatives **13α** and **13β**. These anomers were readily isolated by column chromatography, and their $J_{3,4}$ values (0 and 4.5 Hz for **13α** and **13β**, respectively) clearly indicate their anomeric configurations.

Treatment of the 3:4 mixture of **13α** and **13β** with sodium hydride in dry ether caused cyclisation and anomerisation, to give a 1:3 mixture of the pyrazole derivatives **14α** and **14β** (the α- and β-anomeric configurations were indicated by the $J_{3,4}$ values of 1 and 4 Hz, respectively). Similar treatment of each pure anomer **13α** and **13β** showed that the *endo*-anomer **13α** reacted the slowest, which results in considerable epimerisation that was not observed (t.l.c.) with the *exo*-anomer **13β**. The lower rate of reaction of **13α** may be due to greater steric interaction in



14 αβ R = OMe
15 αβ R = NH_2

the cyclisation step. When the pure anomers **14α** and **14β** were epimerised in methanolic 0.1M sodium methoxide, **14β** was found to be thermodynamically the most stable. This finding contrasts with the higher stability of **5α** and several other *endo*-analogues[4], and may indicate a stronger interaction of the *endo*-pyrazole group in **14α**, in comparison with the substituted methylene group CH_2R of **5α** and similar compounds, which results in the bulky R group adopting an *exo* orientation. However, electronic effects accompanying the formation of an anionic aglycon[4] cannot be ruled out.

The absence of epimerisation during the cyclisation of **13β** may be due to the high rate of reaction, the stability of the product **14β**, and the separation of the product during the reaction.

The anomers **14α** and **14β** were isolated by column chromatography (*cf.* ref. 3b) and, when treated separately with saturated methanolic ammonia, yielded the amides **15α** and **15β**, respectively; **14α** but not **14β** underwent some epimerisation in this reaction. Hydrolysis of **15α** and **15β** yielded pyrazofurin (**2β**) and pyrazofurin B (**2α**), respectively.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Evaporations were conducted *in vacuo* at <40° (bath). Elemental analyses were carried out by the Microanalysis Service of the University of Granada. Optical rotations were measured with Perkin–Elmer 141 and 241 polarimeters, using a 10-cm standard cell. I.r. spectra were recorded with a Beckman Aculab IV spectrophotometer. ^{1}H-n.m.r. spectra of **5α** and **5β** were recorded with a Bruker WM 250 SY instrument. A Perkin–Elmer–Hitachi R-24B (60 MHz) spectrometer (locked on the signal of internal Me_4Si) was used for **7** and **13–15**. Coupling constants were measured directly from the spectra. The mass spectra for **5α** and **5β** were obtained by using a Kratos MS-25 spectrometer. U.v. spectra were recorded with a Beckman DB-GT spectrophotometer. T.l.c. was performed on Kieselgel 60 F_{254} (Merck) and detection was effected by u.v. light. Flash liquid–column chromatography was performed on Kieselgel 60 (Merck, 230–400 mesh).

Methyl 4-(2,3-O-isopropylidene-5-O-trityl-α- and -β-D-ribofuranosyl)-3-oxobutanoate (**5α** *and* **5β**). — A solution of 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranose (**3**; 12.45 g, 28.7 mmol), 3-methoxycarbonylacetonylidenetriphenylphosphorane (**4**; 20.8 g, 55 mmol), and benzoic acid (0.2 g, 1.6 mmol) in anhydrous benzene (200 mL) was boiled under reflux until t.l.c. (hexane–ethyl acetate, 2:1) showed the absence of **3** (54 h). The solvent was evaporated, the residue was extracted with hexane–ethyl acetate (3 × 100 mL, 4:1), and the combined extracts were concentrated. To a solution of the resulting syrup in ethyl acetate (30 mL) was added hexane (30 mL), which caused the separation of an oil that was removed. The hexane–ethyl acetate extracts were combined and concentrated, and the residue was subjected to flash chromatography (hexane–ethyl acetate, 6:1), yielding

a mixture (12.92 g, 84.9%) of **5α**, **5β**, **6α**, and **6β** in the ratios 12:3:2:1 (^{1}H-n.m.r. data), R_F 0.56 (ethyl ether–hexane, 3:2), $[\alpha]_D^{20}$ +3° (*c* 0.8, methanol); ν_{max}^{KBr} 3050, 3020, 2970–2920, 1745, 1715, 1375, 890, 755, and 740 cm^{-1}; λ_{max}^{MeOH} 216 nm (ε 12,700). Mass spectrum: *m/z* 530 (M^+), 515 ($M^+ - CO_2Me$), 453 ($M^+ - Ar$), 287 ($M^+ - Ph_3C$).

Anal. Calc. for $C_{32}H_{34}O_7$; C, 72.43; H, 6.45. Found: C, 72.29; H, 6.72.

Methyl 2-diazo-4-(2,3-O-isopropylidene-5-O-trityl-α- and -β-D-ribofuranosyl)-3-oxobutanoate (**13α** *and* **13β**). — Triethylamine (0.47 g, 46.4 mmol) was added to a well-stirred solution of the foregoing mixture of **5** and **6** (2.42 g, 4.64 mmol) in acetonitrile (7.4 mL, 4.64 mmol) at 15°, and tosyl azide (0.91 g, 4.64 mmol) was then added portion-wise. The mixture was allowed to attain room temperature and stirring was continued for 2.5 h, the solvents were evaporated, and the residue was triturated with ether (10 mL). The ethereal solution was washed successively with aqueous KOH (0.3 g in 30 mL, then 0.05 g in 25 mL) and water (25 mL), dried (Na_2SO_4), filtered, and concentrated to yield a mixture of **13α** and **13β** as a yellow foam (2.48 g, 96%). Column chromatography (hexane–ethyl acetate, 6:1) on silica gel yielded **13α** and **13β** in the ratio 3:4.

Compound **13α**, R_F 0.68 (hexane–ethyl acetate, 2:1), had m.p. 61° (from hexane–ethyl acetate), $[\alpha]_D^{20}$ +2° (*c* 0.8, methanol); ν_{max}^{KBr} 3045–3020, 2980–2925, 2130, 1740, 1650, 1375, 860, 760, and 740 cm^{-1}; λ_{max}^{MeOH} 255 nm (ε 10,500) and 227 nm (ε 16,600).

Anal. Calc. for $C_{32}H_{32}N_2O_7$: C, 69.05; H, 5.79; N, 5.03. Found: C, 69.20; H, 6.02; N, 4.93.

Compound **13β**, R_F 0.64, had m.p. 54° (from hexane–ethyl acetate), $[\alpha]_D^{20}$ −1° (*c* 0.8, methanol); ν_{max}^{KBr} 3075–3030, 2940, 2140, 1725, 1715, 1660, 1380, 865, 765, and 745 cm^{-1}; λ_{max}^{MeOH} 257 (ε 12,300) and 232 nm (ε 13,500).

Anal. Found: C, 68.90; H, 5.71; N, 5.12.

4-Hydroxy-3-(2,3-O-isopropylidene-5-O-trityl-α- and -β-D-ribofuranosyl)-5-methoxycarbonylpyrazole (**14α** *and* **14β**). — Sodium hydride (0.231 g, 9.65 mmol) was added to a well-stirred solution of a mixture (4.29 g, 7.71 mmol) of **13α** and **13β** in dry ether (30 mL). The mixture was stored for 4 h at room temperature and then boiled under reflux for 30 min. The excess of NaH was destroyed with MeOH, the mixture was neutralised with dilute HCl and concentrated *in vacuo*, and the residue was subjected to column chromatography (hexane–ethyl acetate 4:1) to yield **14α** and **14β**.

Compound **14α** (0.557 g, 12.96%), R_F 0.52 (hexane–ethyl acetate, 1:1), had m.p. 56° (from hexane), $[\alpha]_D^{20}$ −2° (*c* 0.9, chloroform); ν_{max}^{KBr} 3500–3200, 3060–3010, 2980–2930, 1730, 1690, 1450, 770, 760, and 740 cm^{-1}; λ_{max}^{MeOH} 267 (ε 5600) and 227 nm (ε 13,300).

Anal. Calc. for $C_{32}H_{32}N_2O_7 \cdot H_2O$: C, 67.35; H, 6.20; N, 4.28. Found: C, 67.40; H, 6.31; N, 4.22.

Compound **14β** (1.46 g, 34.03%), R_F 0.6 (hexane–ethyl acetate, 1:1), had m.p. 67° (from hexane), $[\alpha]_D^{20}$ −10° (*c* 0.5, chloroform); ν_{max}^{KBr} 3380, 3300, 3060,

3030, 2980, 2910, 1730–1680, 760, and 740 cm^{-1}; λ_{max}^{MeOH} 267 (ε 5500) and 225 nm (ε 13,000).

Anal. Calc. for $C_{32}H_{32}N_2O_7$: C, 69.05; H, 5.79; N, 5.03. Found: C, 68.16; H, 6.03; N, 4.64.

When **13α** was treated as described above, t.l.c. (hexane–ethyl acetate, 1:1) of the products revealed a 1:1 mixture of **14α** and **14β**. When **13β** was treated as described above, t.l.c. revealed **14β** as the sole product.

When **14α** was treated with methanolic 0.1M sodium methoxide at room temperature for 24 h, t.l.c. (hexane–ethyl acetate, 1:1) revealed a 2:3 mixture of **14α** and **14β**, but under these conditions **14β** was unchanged.

4-Hydroxy-3-(2,3-O-isopropylidene-5-O-trityl-α-D-ribofuranosyl)pyrazole-5-carboxamide (**15α**). — A solution of **14α** (0.27 g, 0.49 mmol) in dry MeOH (15 mL) was saturated with anhydrous ammonia at 15°, and the solution was heated in a sealed tube for 3 h at 95° and then cooled. After removal of the solvent under reduced pressure, the residue was subjected to chromatography on silica gel (hexane–ethyl acetate, 1:1) to yield **15α** (0.155 g, 58.35%), R_F 0.45 (ethyl acetate–hexane, 2:1), which had m.p. 42° (from hexane), $[\alpha]_D^{20}$ −15° (*c* 1.2, chloroform); ν_{max}^{KBr} 3440, 3300, 3060, 2980, 2930, 1660, 1610, 1300, 1155, 760, and 740 cm^{-1}; λ_{max}^{MeOH} 264 (ε 5300) and 228 nm (ε 13,500).

Anal. Calc. for $C_{31}H_{31}N_3O_6$: C, 68.74; H, 5.76; N, 7.75. Found: C, 68.92; H, 5.81; N, 7.78.

4-Hydroxy-3-(2,3-O-isopropylidene-5-O-trityl-β-D-ribofuranosyl)pyrazole-5-carboxamide (**15β**). — A solution of **14β** (1 g, 1.79 mmol) in dry MeOH (20 mL) was saturated with anhydrous ammonia at 15°, heated in a sealed tube for 8 h at 95°, and then worked-up as described for **15α** to give **15β** (0.80 g, 82.21%), R_F 0.47 (ethyl acetate–hexane, 2:1), which had m.p. 71° (from hexane), $[\alpha]_D^{20}$ −25° (*c* 0.8, methanol); ν_{max}^{KBr} 3440, 3300, 3200, 3060, 3030, 2980, 2930, 1660, 1610, 1375, 1155, 760, and 740 cm^{-1}; λ_{max}^{MeOH} 262 (ε 6060) and 225 nm (ε 12,500).

Anal. Calc. for $C_{31}H_{31}N_3O_6$: C, 68.74; H, 5.76; N, 7.75. Found: C, 68.72; H, 5.70; N, 7.82.

4-Hydroxy-3-(α-D-ribofuranosyl)pyrazole-5-carboxamide (**2α**, *pyrazofurin B*). — To a solution of **15α** (0.4 g, 0.74 mmol) in anhydrous MeOH (2 mL) was added a solution (2 mL) of anhydrous MeOH saturated at 15° with HCl. The mixture was stored at room temperature for 30 min and then concentrated at low temperature. The resulting syrup was partitioned between chloroform and water (30 mL, 1:1). The aqueous solution was neutralised with Lewatit MP-62 (HO^-) resin, filtered, and concentrated at 0°. The residue was purified by column chromatography on silica gel (ethyl acetate–acetone–methanol–water, 6:1:1:1) to give **2α** (0.108 g, 87%), m.p. 69–70° (from water), $[\alpha]_D^{20}$ −7.6° (*c* 0.8, water), the i.r. spectrum of which was identical to that of a reference sample.

4-Hydroxy-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide (**2β**, *pyrazofurin*). — Treatment of **15β** (0.5 g, 0.92 mmol), as described above for **15α**, gave **2β** (0.136 g, 88%), m.p. 110–113° (from water), $[\alpha]_D^{20}$ −45.8° (*c* 0.8, water), the i.r. spectrum of which was identical to that of authentic sample.

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