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THE STRUCTURES OF 9-HYDROXYZINNOLIDES AND THEIR REARRANGED ACETATES*

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Key Word Index—*Zinnia acerosa, Z juniperifolia, Z peruviana*, Compositae, elemanolides, 9β-hydroxyzinnolides, δ-lactones, juniperin, 11,13-dehydrozinarosin

Abstract—Eleman-8β,12-olides containing a β-hydroxyl group at C-9 exist in equilibrium with 8β-hydroxyeleman-9,12-olides Acetylation of 9β-hydroxyelemanolides gave a mixture of 8β- and 9β-acetyl derivatives in which the former predominates The structures of zinaflorin II acetate, zinaflorin III and other 9β-hydroxyelemanolide acetates have been revised The absolute stereochemistry of the δ-lactone juniperin has been established by means of an X-ray crystallographic analysis

INTRODUCTION

Previous studies of the genus *Zinnia* showed the presence of elemanolides with different combinations of ester and hydroxyl functions at C-6 and C-9 [1, 2] Elemanolides with an acetal function (**1**) [3, 4] and δ-elemanolides (**2**) were also found as constituents of this genus [5, 6]

We recently established the structures of zinaflorins I and II [4] as **3a** and **3b** The structure of zinaflorin II acetate not only requires a change in stereochemistry, but also a major revision of the reported structure [7] is required, since its spectra show large unexpected differences when compared with those of zinaflorin II The structure of zinaflorin III [7], whose main spectroscopic features are similar to those of zinaflorin II acetate, should also be revised

RESULTS AND DISCUSSION

The acetylation product of zinaflorin II, when prepared at steam bath temperature, no longer exhibited the characteristic bands for a γ-lactone in its IR spectrum Instead, a new band at 1735 cm^{-1} (δ-lactone) arose in conjunction with the acetate band, thus indicating the expansion of the γ-lactone into a δ-lactone This change was also evident in the ^1H NMR spectrum, which showed the exocyclic methylene signals as singlets The signal due to H-8 was shifted downfield (δ5 50, J = 8, 4 Hz) The H-9 signal, which in zinaflorin II appeared as a doublet, had in its acetate changed to a doublet of doublets due to long-range coupling with H-7, indicating a *W*-arrangement between H-9 and H-7 This established the stereochemistry of the proton at C-9 as α since it should have the same orientation as H-7 The values of H-8 coupling constants indicated an α disposition of this proton, therefore the structure of zinaflorin II acetate should be represented by **4a** rather than **3c** [7]

If the above arguments are extended to zinaflorin III, whose spectroscopic characteristic are quite similar to those of zinaflorin II acetate (**4a**), its structure should be as represented by **4b**

The ^1H NMR spectrum of the natural δ-lactone juniperin (**4c**) [5] is similar (except for the ester signals) to those of zinaflorin II acetate and zinaflorin III (see Table 1)

To establish the stereochemistry of juniperin in every chiral centre a crystallographic analysis was performed The resulting structure is depicted in **4c**, and its perspective molecular drawing is shown in Fig 1 This figure represents the absolute configuration if H-7 is α as in all sesquiterpene lactones of authenticated stereochemistry Final positional and equivalent isotropic parameters for non-H atoms are listed in Table 2

The established structure and stereochemistry for juniperin **4c** also confirm the structures of zinaflorin II acetate and zinaflorin III as **4a** and **4b** respectively, since their spectroscopic data are quite similar except for the ester groups at C-6 and C-9

Having demonstrated the drastic transformations of the 9β-hydroxyelemanolides upon acetylation, we submitted zinaflorin IV (**1a**) of known structure [4] to the same acetylation conditions The product was the rearranged acetate **7**

On examination of the chemical literature for other 9-hydroxyzinnolides and their acetylation products, we found that 9-hydroxy-6-angeloyloxyzinamultifloride (**5a**) [2] afforded, upon acetylation, a product whose ^1H NMR signals correspond to the rearranged acetate **6a**

The acetylation of zinaflorin II (**3b**), when carried out at room temperature, afforded two products The minor one was characterized as the unrearranged acetate **3c** since it showed IR bands at 1772, 1720, 1692 and 1635 cm^{-1} (γ-lactone, acetate and double bonds, respectively) Its ^1H NMR spectrum (Table 1) showed the expected signals

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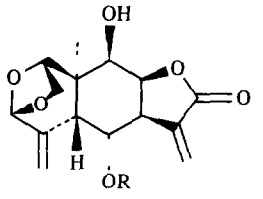
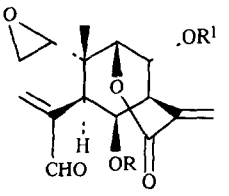


1a R=Ang



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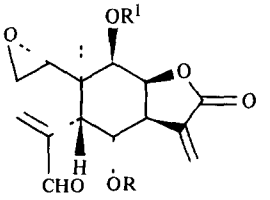
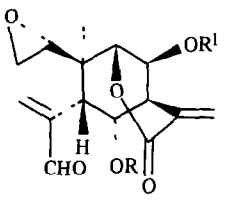
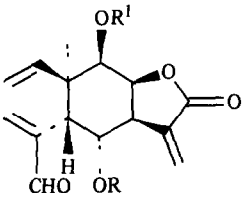


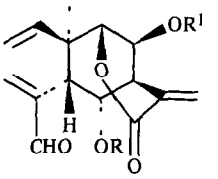
3a R=R^1=Ang
3b R=Ang, R^1=H
3c R=Ang, R^1=Ac



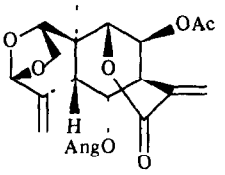
4a R=Ang, R^1=Ac
4b R=Meacr, R^1=Ac
4c R=α-OHiBu, R^1=Ang
4d R=Ang, R^1=H



5a R=Ang, R^1=H
5b R=iBu, R^1=H
5c R=iBu, R^1=Ac



6a R=Ang, R^1=Ac
6b R=iBu, R^1=Ac



7

for an acetate with structure **3c** The major product was the 8β-acetyl-eleman-9,12-olide (**4a**)

Approximately the same results were obtained upon acetylation at room temperature of the new 9-hydroxy-elemanolide-11,13-dihydrozinarosin (**5b**) When this constituent of *Z acerosa* was treated with pyridine–acetic anhydride at room temperature, a mixture of two acetates (**6b** and **5c**) were obtained in a ratio of 2 1

These experiments can be explained by assuming the existence of an equilibrium between 9β-hydroxy-γ-lactones and 8β-hydroxy-δ-lactones in the elemanolide series This assumption is shown to be correct on the following grounds the ^{1}H NMR spectrum of zinaflorin II in $CDCl_3$ (Table 1) is in complete agreement with structure **3b** However, when the spectrum was run in C_6D_6 solution containing two drops of $CDCl_3$ (heating was necessary to dissolve the sample) it showed strong additional signals The new spectral trace was equivalent to the combined spectra of zinaflorin II and the 8β-hydroxy-δ-lactone (**4d**) in a ratio of 3 17

When the solvent was evaporated and the ^{1}H NMR spectrum of the same sample was run in $CDCl_3$, no change was observed When the solution was kept at room temperature for 5 days and the spectrum run again, it showed an increase in the signals corresponding to the γ-lactone In the IR spectrum, the band at 1775 cm^{-1} had increased to an estimated δ γ ratio of 2 1 This solution, which maintained the same δ γ relationship for 10 days, was then percolated through silica gel, to give a mixture whose IR spectrum showed the γ-lactone as the main component, since the IR band at 1775 cm^{-1} had grown larger than that at 1735 cm^{-1} (δ-lactone)

The above experiment indicates the existence of an equilibrium as shown in Scheme 1 Similar equilibria are found in the pseudo-guaianolides linearifolins A and B [8] Formation of the γ-lactone is favoured in the pseudo-guaianolides and the δ-lactone in elemanolides, especially at higher temperatures

EXPERIMENTAL

Acetylation of zinaflorin II (**3b**) A soln of **3b** (74 mg) in pyridine (1 ml) and Ac_2O (1 ml) was left overnight at room temp The reaction mixture was worked up in the usual manner and separated by prep TLC (hexane–$CHCl_3$–EtOAc, 2 2 1, 3 ×) affording 22 mg **3c** and 37 mg **4a** **3c** colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1772, 1720, 1692, 1635, MS *m/z* (rel int) 418 $[M]^+$, 389 $[M-CHO]^+$, 376 $[M-C_2H_2O]^+$, 335 $[M-C_5H_7O]^+$, 318 $[M-C_5H_8O_2]^+$, 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (5 5) **4a** mp 167–170°, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1735, 1725, 1693, 1635, 1625, MS *m/z* (rel int) 418 $[M]^+$, 389 $[M-CHO]^+$, 376 $[M-C_2H_2O]^+$, 358 $[M-HOAc]^+$, 318 $[M-C_5H_8O_2]^+$, 276 $[318-C_2H_2O]^+$, 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (8 6)

Acetylation of zinaflorin II (**3b**) A soln of **3b** (116 7 mg) in pyridine (1 ml) and Ac_2O (1 ml) was left for 4 hr on a steam bath Usual work-up yielded 90 4 mg **4a**

Acetylation of zinaflorin IV (**1a**) A soln of **1a** (51 7 mg) in pyridine (0 5 ml) and Ac_2O (0 5 ml) was left on a steam bath for 0 5 hr The usual work-up gave 17 1 mg acetate **7**, mp 156–158° (Me_2CO–hexane) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1735, 1660, 1640, 1625, MS *m/z* (rel int) 418 $[M]^+$, 375 $[M-C_2H_3O]^+$, 358 $[M-HOAc]^+$, 318 $[M-C_5H_8O_2]^+$, 83 $[C_5H_7O]^+$ (100)

Table 1 ^{1}H NMR spectral data of compounds 3–7 (80 MHz, $CDCl_3$, TMS as internal standard)

H	3b	3c	4a	4b	4c	4d	4d*	5b	5c	6b	7
1	3 06 *t* 3 5	2 84 *t* 3	3 07 *dd* 3 5, 3	3 08 *dd* 3 5, 3	3 08 *dd* 3 5, 3	3 10 *dd* 4, 3	3 02 *t* 3 5	5 84 *dd* 18, 11	5 6 *dd* 17, 11	5 85 *dd* 17, 11	3 76 *dd* 11, 5
2	2 6 *m*†	2 45 *m*†	2 47 *m*†	2 50 *m*†	2 50 *m*†	2 48 *m*†	2 19 *d*† 3 5	5 07 *dd* 11, 1 5	5 04 *d* 11	5 08 *d* 11	4 40 *d* 11
2′								4 96 *dd* 18, 1 5	4 98 *d* 17	4 93 *d* 17	4 16 *d* 5
3	6 58 *s*	6 57 *s*	6 57 *s*	6 61 *s*	6 71 *s*	6 55 *s*	6 11 *s*	6 47 *s*	6 56 *s*	6 63 *s*	5 02 *d* 2
3′	6 21 *s*	6 21 *s*	6 2 *s*	6 25 *s*	6 28 *s*	6 17 *s*	5 46 *s*	6 14 *s*	6 21 *s*	6 19 *s*	4 57 *d* 2
5	3 74 *d* 4	3 59 *d* 4	3 40 *d* 4	3 41 *d* 3	3 43 *d* 3	3 35 *d* 3 5	3 35 *d* 3 5	3 68 *d* 4	3 72 *d* 4	3 41 *d* 3 5	2 82 *d* (*br*) 3
6	5 42 *dd* 4, 3	5 25 *dd* 4, 3	5 00 *dd* 4, 3	5 03 *dd* 4, 3	4 92 *dd* 4, 3	4 99 *t* 3 5	4 90 *t* 3 5	5 24 *dd* 4, 2 5	5 35 *dd* 4, 3	4 88 *t* 3 5	5 31 *t* 3
7	3 4 *m*	3 43 *m*	3 34 *m*	3 35 *m*	3 32 *m*	3 30 *m*	2 95 *m*‡	3 32 *m*	3 34 *m*	3 28 *m*	3 33 *m*
8	4 84 *dd* 8, 4	4 95 *dd* 8, 4	5 50 *dd* 4, 2 5	5 51 *dd* 4, 2 5	5 47 *dd* 3 5, 2 2	4 43 *dd* (*br*) 3 5, 2 5	3 95 *br*	4 83 *dd* 8, 4	4 97 *dd* 8, 4	5 48 *dd* 4, 2 5	5 5 *dd* 4, 2
9	4 01 *d* 4	5 41 *d* 4	4 62 *dd* 2 5, 2	4 65 *dd* 2 5, 2	4 62 *dd* 2 2, 2	4 55 *t* 2 5	4 21 *t* 2 5	3 77 *d* 4	5 21 *d* 4	4 35 *t* 2 5	4 55‡
13	6 28 *d* 3 5	6 37 *d* 3 5	6 75 *s*	6 78 *s*	6 76 *s*	6 76 *s*	6 58 *s*	6 25 *d* 3 5	6 33 *d* 3 5	6 72 *s*	6 20 *s* (*br*)
13′	5 70 *d* 3	5 79 *d* 3	5 87 *s*	5 88 *s*	5 94 *s*	5 89 *s*	5 45 *s*	5 71 *d* 3	5 77 *d* 3	5 83 *s*	5 88 *s* (*br*)
14	1 01 *s*§	1 05 *s*§	1 14 *s*§	1 14 *s*§	1 16 *s*§	1 08 *s*§	1 27 *s*§	1 34 *s*§	1 40 *s*§	1 44 *s*§	1 44 *s*§
15	9 42 *s*	9 38 *s*	9 33 *s*	9 40 *s*	9 35 *s*	9 31 *s*	9 32 *s*	9 38 *s*	9 28 *s*	5 42 *s*	9 38 *s*
OCOR	6 15 *q* (*br*) 8	6 20‡	6 20‡	6 20 *br*	1 56‖	6 18‡	5 86 *q* (*br*) 7	2 55 *h* 7	2 67 *h* 7	2 67 *h* 7	6 15 *q* (*br*) 8
	1 98 *d* (*br*)§ 8	2 03 *d*§ 8	2 01‖	5 88 *br*	6 17 *q* (*br*) 7	2 05 *d* (*br*)§ 7	1 94 *d* (*br*) 7	1 17 *d*§ 7	1 19 *d*§ 7	1 18 *d*§ 7	2 01 *d* (*br*)§ 8
	1 93 *s* (*br*)§	1 97 *br*§	2 08§	2 03 *s*§	1 92 *d* (*br*)§ 7	1 99 *br*§	1 73 *br*§	1 15 *d*§ 7	1 17 *d*§ 7	1 15 *d*§ 7	1 86 *br*§
		2 1 *s*§		2 08 *s*§	1 82 *br*§				2 03 *s*§	2 05 *s*§	2 05 *s*§

*Run in C_6D_6
†Intensity two protons
‡Superimposed signal
§Intensity three protons
‖Intensity six protons

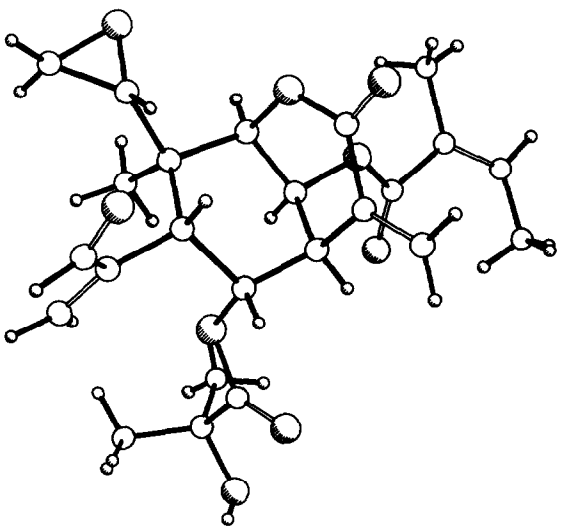
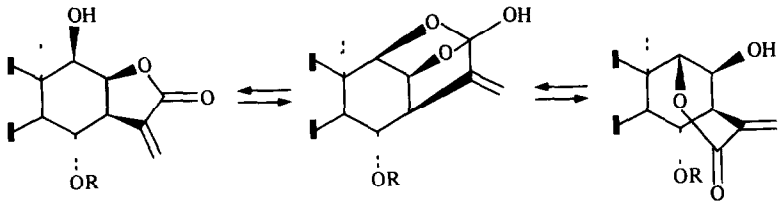


Fig 1

Z acerosa The whole plant (520 g), collected in San Luis Potosí, México, in September 1981 (voucher on deposit at the Herbarium of the Instituto de Biología, UNAM, MEXU 371498 was extracted with $CHCl_3$ The solvent was evapd and the residue (16 1 g) chromatographed in a column containing 300 g silica gel Elution with $CHCl_3$–Me_2CO (19 1) afforded crude 11,13-dehydrozinarosin (**5b**) After CC purification, 1 5 g **5b** was obtained as a colourless oil $C_{19}H_{24}O_6$, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3520, 1765, 1732, 1725, 1690, 1635, 1625

Acetylation of 11,13-*dehydrozinarosin* (**5b**) A soln of **5b** (203 mg) in pyridine (2 ml) and Ac_2O (2 ml) was allowed to stand overnight at room temp and then worked up to give a mixture of **6b** and **5c**, which was separated by prep TLC ($CHCl_3$–Me_2CO, 93 7) Compound **5c**, (43 mg) crystallized from Me_2CO–*i*-Pr_2O, mp 154–155° IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1770, 1740, 1730, 1690, 1640; MS *m/z* (rel int) 390 $[M]^+$, 361 $[M-CHO]^+$, 331 $[M-OAc]^+$, 319 $[M-C_4H_7O]^+$, 303 $[M-C_4H_7O_2]^+$, 43 (100) Compound **6b** (95 mg), mp 168–170° (Me_2CO–*i*-Pr_2O). IR ν_{max}^{film} cm^{-1} 1745, 1735, 1690, 1640, 1630, MS *m/z* (rel int) 390 $[M]^+$, 361 $[M-CH_2OH]^+$, 348 $[M-C_2H_2O]^+$, 330 $[M-HOAc]^+$, 320 $[M-C_4H_6O]^+$, 302 $[M-C_4H_8O_2]^+$, 43 (100)

X-Ray analysis of juniperin Single colourless prisms of juni-



Scheme 1

Table 2 Atomic coordinates ($\times 10^4$) and equivalent isotropic temperature factors ($A^2 \times 10^3$) for juniperine

Atom	x	y	z	U_{eq}*
C-1	6584 (3)	5193 (3)	10004 (3)	50 (1)*
C-2	5727 (4)	4629 (3)	10370 (4)	67 (2)*
C-3	5994 (4)	4614 (3)	7176 (3)	64 (2)*
C-4	6863 (4)	4746 (2)	7746 (3)	48 (1)*
C-5	7174 (3)	5615 (2)	8265 (3)	41 (1)*
C-6	7405 (3)	6380 (2)	7517 (3)	40 (1)*
C-7	7804 (3)	7234 (3)	8031 (3)	44 (1)*
C-8	6981 (3)	7527 (2)	8819 (3)	44 (1)*
C-9	6900 (3)	6798 (3)	9594 (3)	44 (1)*
C-10	6458 (3)	5906 (3)	9179 (3)	42 (1)*
C-11	8899 (3)	7104 (3)	8531 (3)	49 (1)*
C-12	8914 (3)	6805 (3)	9596 (3)	52 (1)*
C-13	9825 (4)	7247 (4)	8085 (3)	75 (2)*
C-14	5233 (3)	6055 (3)	8920 (3)	52 (1)*
C-15	7585 (4)	3978 (3)	7881 (4)	71 (2)*
C-16	6564 (3)	6918 (3)	6020 (3)	47 (1)*
C-17	5464 (4)	7121 (3)	5532 (3)	55 (1)*
C-18	4864 (5)	7796 (5)	6159 (4)	105 (3)*
C-19	4832 (5)	6294 (4)	5382 (5)	98 (2)*
C-20	7141 (4)	9112 (3)	8910 (4)	60 (1)*
C-21	7442 (4)	9858 (3)	9624 (4)	63 (2)*
C-22	7837 (5)	10600 (4)	9286 (4)	80 (2)*
C-23	8048 (7)	10835 (4)	8261 (4)	109 (3)*
C-24	7238 (5)	9684 (4)	10737 (3)	76 (2)*
O-1	6090 (3)	5376 (2)	10942 (2)	72 (1)*
O-2	6431 (2)	6574 (1)	6951 (2)	43 (1)*
O-3	7342 (2)	8321 (2)	9339 (2)	54 (1)*
O-4	7962 (2)	6652 (2)	10074 (2)	51 (1)*
O-5	9740 (2)	6704 (2)	10071 (3)	74 (1)*
O-6	8349 (3)	3941 (2)	8448 (3)	85 (1)*
O-7	7425 (2)	7043 (2)	5644 (2)	68 (1)*
O-8	5672 (3)	7545 (2)	4591 (2)	59 (1)*
O-9	6740 (3)	9170 (2)	8074 (3)	76 (1)*

*$U_{eq} = (U_{11} \times U_{22} \times U_{33})^{1/3}$

perine grown by slow evapn of an EtOAc soln proved to be suitable for X-ray analysis

Initial photographic studies showed the mmm Lave symmetry and systematic absences in $h00$ with h = odd, $0k0$ with k = odd and $00l$ with l = odd, thus uniquely defining the space group as $P2_12_12_1$ Unit cell dimensions were obtained by a least-squares fit to the angular settings of 15 centred reflections on a Nicolet R3m diffractometer equipped with a graphite monochromator crystal Crystal data for juniperin $C_{24}H_{30}O_9$, M_r 462 5, a = 12 279 (3), b = 14 901 (5), c = 13 324 (3) Å, V = 2437 8 (3) $Å^3$, d_{calcd} = 1 25 g/cm^3, Z = 4, space group $P2_12_12_1$, $\mu(CuK_\alpha)$ = 7 67 cm^{-1} The crystal chosen for intensity measurement had the dimensions 0 24 × 0 24 × 0 32 mm, and was mounted approximately along the c axis on a glass fiber Intensity measurements were made with CuK_α (λ = 1 5418 Å) radiation utilizing the ω-scan technique, the rate of scanning being varied from 4 0 to 29 3 deg/min^{-1} Two reflections were routinely monitored at intervals of 100 measurements All reflections in the hkl octant according to $3° < 2\theta < 115°$ ($\sin\theta/\lambda$ = 0 550 $Å^{-1}$) were collected The total number of data collected was 1929, of which 1711 reflections had $I > 2\,0\sigma(I)$ and these formed the basis of the structural solution and refinement, these reflections were corrected for Lorentz and polarization effects, no absorption correction was applied The crystal structure was solved by direct methods using the program package SHELXTL [9] The trial structure was refined by a blocked cascade least-squares procedure with anisotropic temperature factors for the non-H atoms and with a fixed isotropic temperature factor, U = 0 06 $Å^{-2}$, for the H atoms bonded to C atoms, the H atoms bonded to O atoms were found in a difference Fourier map The function minimized was $\Sigma\omega([Fo]-[Fc])^2$ with a weighting scheme $\omega = [\sigma^2(Fo) + G(Fo)^2]^{-1}$, where σ is the standard deviation of the observed amplitudes, based on counting statistics, and G is a variable to be adjusted after each cycle, final G = 0 0026, maximum shift of parameters in the last cycle 0 2σ, no peaks > 0 3 e/$Å^{-3}$, anomalous dispersion corrections were applied to the scattering factors for the O and C atoms, atomic scattering factors were from *International Tables for X-Ray Crystallography* [10], isotropic extinction parameter X = 0 0047, final R = 0 047, R_ω = 0 058 ($R_\omega = \Sigma\omega^{1/2}([Fo]-[Fc])/\Sigma\omega^{1/2}[Fo]$)

All computations were performed in the laboratory on a Nova 4S computer and plots were drawn on a Tektronix plotter

A list of the observed and calculated structure factors, anisotropic thermal parameters and atomic coordinates has been deposited at the Cambridge Crystallographic Centre

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