Tetrahedron Vol 45, No 6, pp 1759 to 1762, 1989
Printed in Great Britain

0040-4020/89 $3 00+ 00

REGIOSPECIFIC C-9 SUBSTITUTION OF ELLIPTICINE DERIVATIVES

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(*Received in UK* 30 *December* 1988)

Abstract - 6-Methylellipticine (6) undergoes acylation at the C(9)-position, under Friedel Crafts reaction conditions. The C(9)-formyl compound (8) rearranges to the corresponding hydroxy derivative (9) upon treatment with hydrogen peroxide, in methanol, in the presence of sulphuric acid. The two steps provide a convenient procedure for the specific C(9)-hydroxylation of the ellipticine template.

The alkaloid ellipticine (1a, Scheme I), isolated from several members of the Ochrosia species[1] and from Strychnos dinklagei Gilg.[2] exhibits broad spectrum antitumour activity in a variety of experimental models, both in vitro and in vivo.[3] Comparison of the biological activities of many derivatives of ellipticine has resulted in different hypotheses concerning its mechanism of action. Evidence has been developed which supports the idea that in vivo hydroxylation at C-9 followed by subsequent oxidation of 1b to an iminoquinone intermediate 2 results in the formation of a strong electrophilic intermediate, which can react with nucleo-bases, sugar-hydroxyls and proteins.[4] The covalent binding of a hydroxylated ellipticine derivative to nucleic acids of L 1210 cells in culture has been demonstrated.[5] Although the intercalating properties of ellipticine-derivatives are well-documented, a correlation between DNA-affinity and cytotoxicity in this series of compounds does not exist.[6]

Of particular interest is the interaction of ellipticine-derivatives with topoisomerase II leading to DNA-cleavage.[7] The ability of ellipticine-derivatives to interfere with the action of topoisomerase II also requires the presence of an oxidizable phenolic group.

It was recognized by us, that a facile method of introducing a hydroxyl group at the C(9)-position of the ellipticine template would be of great practical value for synthesizing new, biologically active ellipticine-derivatives. This communication describes such a method.

To date, in most cases, the synthesis of 9-substituted ellipticine derivatives has been achieved via construction of the ellipticine-nucleus starting from appropriately substituted precursors. Direct introduction of the 9-hydroxy group in the ellipticine-nucleus was accomplished by Dat-Xuong and coworkers in a multistep procedure, consisting of bromination, amination, diazotation, and hydrolysis of the resulting diazonium salt.[8]

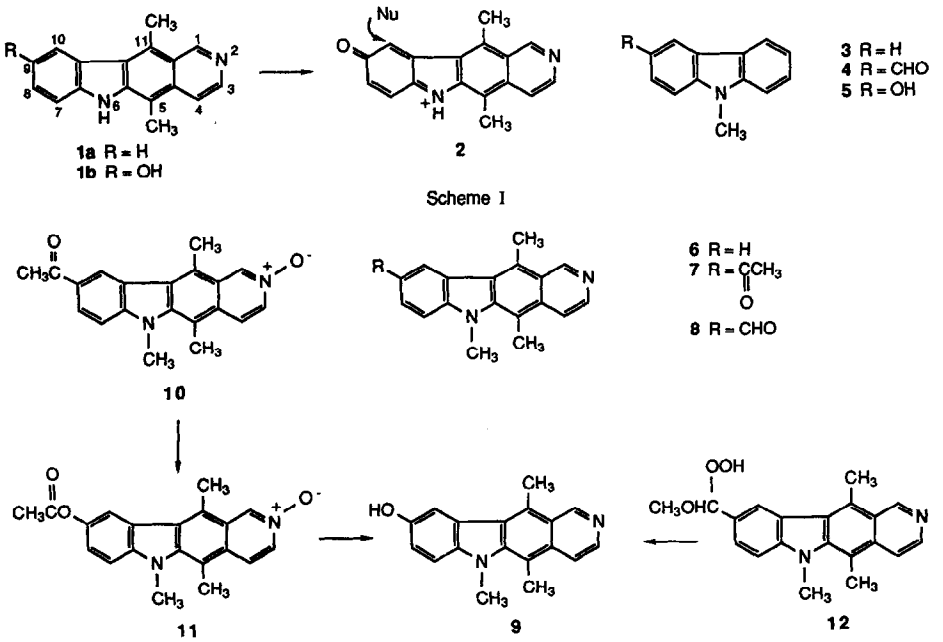
The strategy of our approach to the 9-hydroxy derivatives of ellipticines was based upon two chemical transformations, namely (i) acylation of the 9-positions of ellipticine and (ii) a Baeyer Villiger rearrangement of the 9-acyl derivative.[9] To test the feasability of this strategy a model study was performed on 9-methylcarbazole (3). The latter could be selectively formulated at the 3-position using Vilsmeyer conditions. A hydrogen peroxide mediated rearrangement of 4 [10] gave 3-hydroxy-9-methylcarbazole (5) in good yields.[11]

Application of the Vilsmeyer reaction to 6-(N)-methylellipticine (6 [12] Scheme II) did not, in our

hands, give satisfactory results, although the formylation product could be identified in low yields, in the reaction mixture. Consequently, we turned our attention to the Lewis acid catalyzed electrophilic substitution of the ellipticine template. The pyridocarbazole nucleus of ellipticine is susceptible to attack, by electrophiles, at centres C(7), C(9) and N(2). Bromination of ellipticine has been reported to yield the 9-bromo derivatives.[8] Our study of the acylation of 6, under Friedel Crafts conditions, shows that substitution occurs exclusively at the C(9)-position. Thus, reaction of 6 with acetyl chloride, in the presence of $AlCl_3$, yielded 9-acetyl-6-methyl-ellipticine (7) (Scheme II). The formylation of 6 at the 9-position could be achieved via an $AlCl_3$ catalyzed reaction with chloromethyl dichloromethyl ether, followed by hydrolysis.[13] The product 8 was obtained in > 90% yield.

In principle, 7 and 8 can give esters of 9-hydroxy-6-methylellipticine (9), via Baeyer-Villiger rearrangements with either hydrogen peroxide or peracids.[12] Treatment of 7 with one equivalent of m-CPBA (m-chloroperbenzoic acid) did not, however, cause the rearrangement reaction. Instead, the main product isolated was the corresponding N-oxide 10. When 10 was subjected to reaction with a second equivalent of m-CPBA, the ester 11 was formed as the sole rearranged product. This is not wholly unexpected, since of the two groups which can potentially migrate in the Baeyer-Villiger rearrangement of 10 the "migrating aptitude" of the tetracyclic heterocyclic aryl moiety will be expected to be far greater than that of the methyl group. Attempts to treat 7 with two equivalents of m-CPBA directly did not proceed satisfactorily. A complex reaction mixture was obtained from which a product, whose structure is as yet undefined, has been isolated.

The conversion of 11 to hydroxy compound 9 could be achieved by further steps involving hydrolysis and deoxygenation of the N-oxide by triphenylphosphine. However, this sequence was laborious compared to the one which could be developed starting from the formyl derivative 8.
When 8 was allowed to react with aqueous hydrogen peroxide under strongly acidic conditions 9-hydroxy-6-methylellipticine 9 was obtained in one practical step, in high yield. During the reaction, nitrogen N-2 is protonated which prevents its transformation to the corresponding N-oxide. Oxidation of aldehyde 8 with hydrogen peroxide in methanol-sulphuric acid might proceed, as suggested by Matsumoto et al.[11] via a peroxy hemiacetal 12 as the reactive intermediate. Under the reaction conditions, 12 can potentially decompose either by hydrogen migration or by aryl migra-



Scheme I

Scheme II

tion. In the present case 9 was the sole product isolated from the reaction mixture, emphasizing once again the ease of aryl C → O migration.

The sequence 6 → 8 → 9 in only two steps constitutes a facile strategy for regioselective introduction of a hydroxyl substituent at the 9-position of the ellipticine template.

Application of the developed methods of functionalization of ellipticine to the synthesis of a variety of substituted ellipticine-derivatives with improved solubility characteristics is being vigourously pursued in our laboratory.

EXPERIMENTAL

General

All melting points are uncorrected. IR spectra were recorded on a Perkin Elmer 1310 spectrophotometer. The absorptions are given in cm^{-1}. PMR spectra were run on Bruker WM 250 and AC 200 instruments. Unless stated otherwise, IR and NMR spctra were taken in $CHCl_3$ and $CDCl_3$, respectively. Mass spectra were obtained with a Varian Matt 711 spectrometer. Thin-layer chromatography was carried out with silica gel F 254 plates.

9-Methylcarbazole (3).

To a suspension of 0.3 g sodium hydride in 10 ml dry dimethyl formamide was added 2.1 g carbazole and after stirring at room temperature, a mixture of 1.8 g methyl iodide and 5 ml DMF was added dropwise. After 1 h of additional stirring, the mixture was diluted with water and extracted three times with ethyl acetate. Combining the extracts, drying and evaporating the solvents afforded 3. The product was recrystallized from ethanol. Yield 79%: mp. 87-88°C (lit. [14]: 88-89°C); PMR: 3.7 (s, 3H, $N-CH_3$), 7.0-7.6 (m, 6H, aromatic protons), 7.1 (d, 2H, J = 7, H-4 + H-5).

3-Formyl-9-methylcarbazole (4)

To a solution of 5.0 g 9-methylcarbazole (3) in 20 ml dry dimethyl formamide was added 5 ml phosphorus oxychloride and the mixture was heated at 100-110°C during 2 h. After cooling, the mixture was poured into a solution of 20 g sodium acetate ($.3H_2O$) in 100 ml water. Extraction with ethyl acetate (250 ml total), washing with water, drying and evaporating the solvents produced a brown oil. Pure 4 was obtained by column chromatography (silica gel, ethyl acetate/hexanes 2:3) and crystallisation from diethyl ether. Yield: 59%; mp. 79-82°C (lit. [10]: 74°C); IR: 3020, 1675, 1620 1590, 1210; PMR: 3.85 (s, 3H, $N-CH_3$), 7.31 (t, 1H, J = 2, H-6), 7.41 (d, 2H, J = 8, H-1 + H-8), 7.53 (t, 1H, J = 8, H-7), 7.98 (d, 1H, J = 8, H-2), 8.11 (d, 1H, J = 8, H-5), 8.55 (s, 1H, H-4), 10.05 (s, 1H, COH).

Nuclear Overhauser Difference spectroscopy:	
irradiation:	signals obtained:
3.85 ($N-CH_3$)	7.41 (d, H-1 + H-8)
10.05 (COH)	8.55 (s, H-4), 7.98 (d, H-2)
8.55 (H-4)	8.11 (d, H-5), 10.05 (s, COH)

3-Hydroxy-9-methylcarbazole (5).

To a suspension of 1.06 g 4 in 25 ml methanol in a nitrogen atmosphere was added 0.6 ml hydrogen peroxyde (35% in water) and 0.1 ml conc. sulphuric acid. After 1 h stirring at room temperature, the yellow solution was diluted with 75 ml water and the product extracted with ethyl acetate. The organic layer was washed twice with 50 ml water and after drying, evaporated to afford 5. Yield: 94%; mp. 145-147°C (lit. [15]: 146-147°C); IR: 3600, 3350, 2940, 1625, 1600, 1580, 1485; PMR: 3.78 (s, 3H, NCH_3), 4.99 (bs, 1H, OH), 7.05 (d, 1H, J = 8, H-2), 7.22 (t, 1H, J = 8, H-6), 7.25 (d, 1H, J = 8, H-1), 7.38 (d, 1H, J = 8, H-8), 7.50 (t, 1H, J = 8, H-7), 7.53 (s, 1H, H-4), 8.01 (d, 1H, J = 8, H-5).

9-Acetyl-6-methylellipticine (7).

1.3 g 6-methylellipticine [12] 6 (5 mmol) was dissolved in 50 ml dry methylene chloride. After addition of 1.33 g (10 mmol) aluminium chloride, the suspension was stirred at room temperature during 10 min. After cooling in an ice-bath a solution of 0.71 ml freshly distilled acetyl chloride (10 mmol) in 50 ml methylene chloride was added dropwise over a period of 1.5 h. The suspension was stirred an additional 2 h at room temperature and then poured into water and carefully neutralized with sat. sodium bicarbonate. The organic layer was collected and the water layer extracted twice with chloroform. The combined extracts were dried and concentrated. Addition of ethyl acetate to the residue afforded 7 as a yellow solid. Yield: 96%. An analytically pure sample was obtained via column chromatography (silica gel, methanol/methylene chloride 4:96); mp. 227-229°C; IR: 1665, 1590, 1455, 1355, 1300, 1260; PMR: 2.67 (s, 3H, $CO-CH_3$), 2.89 (s, 3H, $C(5)-CH_3$), 3.07 (s, 3H, $C(11)-CH_3$), 3.98 (s, 3H, $N-CH_3$), 7.24 (d, 1H, J = 8.6, H-7), 7.79 (d, 1H, J = 6.0, H-4), 8.10 (dd, 1H, J = 8.6, J = 1.6, H-8), 8.47 (d, 1H, J = 6.0, H-3), 8.74 (d, 1H, J = 1.5, H-10), 9.59 (s, 1H, H-1). MS: m/z = 302.1422 (calc. for $C_{20}H_{18}N_2O$: 302.1419).

9-Formyl-6-methylellipticine (8).

A suspension of 6.0 g aluminium chloride (45 mmol) in 225 ml dry methylene chloride was stirred at room temperature for 10 min. 5.85 g 6-methylellipticine 6 (22.5 mmol) was added and the mixture stirred an additional 10 min at room temperature. After cooling to 0°C a mixture of 4 ml dichlo-

romethyl methyl ether (45 mmol) and 80 ml methylene chloride was added dropwise over a period of 1.5 h. The mixture was stirred at 0°C another 1.5 h, diluted with 100 ml water and poured into a mixture of 1000 ml water and 600 ml chloroform. Solid sodium carbonate was added until pH = 8 was reached and the layers were separated. The waterlayer was washed twice with 300 ml chloroform, and the combined layers were dried (sodium sulphate) and concentrated. Addition of ethyl acetate to the residue produced 5.3 g 8 as a yellow solid. After concentrating the mother liquor, addition of 10 ml ethyl acetate produced another 0.64 g of product. Total yield: 91%; mp. 223-226°C; IR: 2960, 1675, 1590, 1355, 1305, 1245; PMR: 2.96, 3.14 (2s, 6H, C(5)-CH_3, C(11)-CH_3), 4.06 (s, 3H, N-CH_3), 7.37 (d, 1H, J = 8.5, H-7), 7.85 (d, 1H. J = 6.2, H-40, 8.03 (dd, 1H, J = 8.5, J = 1.4, H-8), 8.52 (d, 1H, J = 6.2, H-3), 8.66 (d, 1H, J = 1.4, H-10), 9.64 (s, 1H, H-1), 10-06 (s, 1H, COH). MS: m/z = 288.1250 (calc. for $C_{19}H_{16}N_2O$: 288.1262).

6-Methyl-9-hydroxyellipticine (9).

4.23 g 8 (15 mmol) was suspended in 300 ml methanol. With stirring, 2.25 ml 95% sulphuric acid, followed by 3.75 ml hydrogen peroxide solution (35%) were added. After refluxing for 5 hours, an additional portion of hydrogen peroxide was added (2 ml). Cooling the mixture and pouring into 200 ml water afforded a red precipitate. After filtration, 200 ml chloroform was added and the mixture was stirred for 0.5 h. Addition of methanol was followed by dropwise addition of a solution of 90 g sodium acetate (.3H_2O) in 100 ml water. Subsequent addition of 1 l chloroform, 100 ml methanol and 500 ml water was followed by separating the layers. The organic layer was dried (Na_2SO_4) and concentrated. Addition of 100 ml ethyl acetate and stirring afforded 3.34 g 9 as yellow crystals. From the filtrate an additional crop of 3.85 g was obtained. Yield: 93%; mp. 300° C (dec.); IR: 3200, 1590, 1475, 1390; PMR (DMSO-d6): 3.00, 3.16 (2s, C(5)-CH_3, C(11)-CH_3), 4.10 (s, 3H, N-CH_3), 7.16 (dd, 1H, J = 8.8, J = 2.0, H-8), 7.52 (d, 1H, J = 8.8, H-7), 7.80 (d, 1H, J = 2.0, H-10), 7.97 (d, 1H, J = 6.7, H-4), 8.39 (d, 1H, J = 6.7, H-3), 9.49 (s, OH), 9.76 (s. H-1).

Oxidation of 9-acetyl-6-methylellipticine (7).

To a solution of 0.151 g 7 (0.5 mmol) in 8 ml dry dichloromethane was added 1.1 mmol m-chloroperbenzoic acid (223 mg 85%). The mixture was stirred in the dark at room temperature. After 20 h according to TLC, the starting material had disappeared. Addition of another 1.1 mmol and additional stirring during 24 h, was followed by addition of sodium bicarbonate solution and extraction with chloroform. The combined chloroform layers were dried (Na_2SO_4) and concentrated. The reaction mixture was purified by column chromatography (silica gel). Elution with dichloro-methane-methanol 97:3 yields the product with undefined structure (12 mg). Increasing the methanol concentration to 6% produces 34 mg of pure 11 and a fraction containing a mixture of 10 and 11, 10: IR: 2960, 1670, 1610, 1590, 1300, 1255, 1180; PMR: 2.62 (s, 3H, $COCH_3$), 2.89, 2.91 (2s, 6H, C(5)-CH_3, C(11)-CH_3), 4.04 (s, 3H, NCH_3), 7.30 (d, 1H, J = 8.6, H-7), 7.84 (d, 1H, J = 7.3, H-4), 8.00 (d, 1H, J = 7.3, H-3), 8.09 (d, 1H, J = 7.7, H-8), 8.66 (s, 1H, H-10), 9.01 (s, 1H, H-1). 11: IR: 2960, 1755, 1590, 1480, 1300, 1180, 1145; PMR: 2.38 (s, 3H, $OCOCH_3$), 2.77, 2.88 (2s, 6H, C(5)-CH_3, C(11)-CH_3), 3.90 (s, 3H, NCH_3), 7.26 (s, 2H, H-7, H-8), 7.78 (d, 1H, J = 7.3, H-4), 7.84 (s, 1H, H-10), 8.06 (d, 1H, J = 7.3, H-3), 9.03 (s, 1H, H-1). MS FAB: $[m+H]^+$ = 335.1401 (calc. for $C_{20}H_{19}N_2O_3$: 335.1396).

ACKNOWLEDGEMENT

The financial support by The Netherlands Foundation for Cancer Research (Koningin Wilhelmina Fonds) and Cedona Pharmaceutical B.V., Haarlem, is greatfully acknowledged. The ellipticine used in this work was supplied by the Natural Products Branch division of Cancer Treatment, NCI, through the courtesy of Dr. M. Suffness.

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