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CHEMISTRY

Synthesis of a Steroidal Conjugate of α-Methylene Lactone, A Potential Anti-tumour Agent

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ABSTRACT:

A novel steroidal α-methylene lactone has been synthesized for selectivity of action on hormone receptive tumours such as breast cancer. The design is based on the coupling of α-methylene lactone, a known alkylating agent with established cytotoxicity, to an estradiol derivative.

KEYWORDS

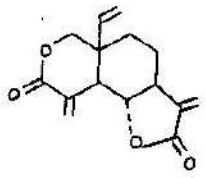
Synthesis, Steroidal α-methylene lactone, hormone receptive tumours.

INTRODUCTION:

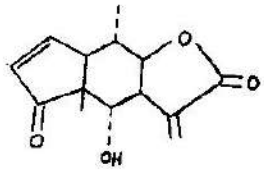
Sesquiterpene lactones such as vernolepin (1) and helenalin (2) have been shown to have cytotoxic effects against animal tumour systems in vivo and in vitro over a wide range of neoplasias. Those of particular interest contain the α-methylene lactone moiety, which combine simplicity in structure with requirements for cytotoxicity, (Lee et al, 1975; Kupchan, 1971). However, the simple low molecular weight lactones have not been very successful in vivo primarily because of low permeability across cell membrane and lack of specificity.

An approach for obtaining selectivity of biologically active alkylating agents (e.g. α-methylene lactone) involve the coupling of alkylating agents to biological carriers. In this form, the biological carrier could be serving a dual role, that is, carrying the alkylating agent to target specific sites and also overcoming cell membrane barriers (because of increase in lipophilicity). In this regard, some steroidal alkylating agents have been synthesized and their activity evaluated against specific cancer cells. These include steroidal mustard alkylating agents (3) and (4) and the steroidal α-methylene lactone (5) (Vollmer et al, 1973; Catsoulacas and Bontis, 1973; Lee et al, 1975). These have been found to have considerable cytotoxic effects against experimental tissues. However, all these lacked specificity of activity.

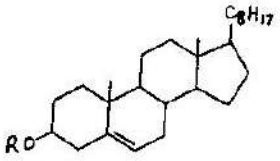
The synthesis of steroidal α-methylene lactone with the objective of having affinity for estrogen receptor and thus being selective and specific have been studied by Chagonda et al (1984). Although the lactones (6) and (7) have high cytotoxicity on tissue culture Hela S_3 cells, they lacked affinity for estrogen receptor.



(1) Vernolepin

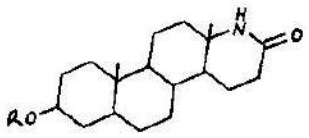


(2) Helenalin

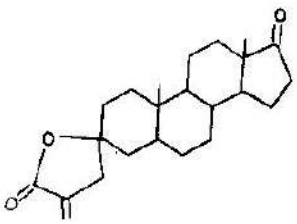


R = $-COCH_2ArN(CH_2CH_2Cl)_2$

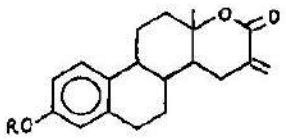
(3)



(4)



(5)



(6) R = H

(7) R = CH_3

Fig.1

Reported here is a rational approach in the design and synthesis of an anti-tumour agent based on the above observations with a view to obtaining target specific agents for hormone receptive tumours such as breast, cervix and prostate.

MATERIALS AND METHODS:

Synthesis:

The melting points were determined on a Reichert hot stage apparatus and are uncorrected. Preparative thin layer chromatograph (TLC) plates were prepared from Kieselgel 60 PF 254 (Merck). IR Spectra were determined with Perkin-Elmer 177 Spectrophotometer. ^1H NMR Spectra were determined in $CDCl_3$ containing TMS at 60 MHz with a Varian EM360A Spectrometer or at 90 MHz with a Perkin Elmer R32 spectrometer. Mass Spectra were recorded on a Kratos MS 80 Mass Spectrometer using a DS-55 data system.

3-Benzyloxy-17-β-hydroxy-estra-1,3,5 (10)-trien-6-one (9):

6-keto estradiol diacetate (8) was obtained from the procedure of Akanni and Marples (1984) in 40% yield. The keto (8) (1g) was suspended in absolute ethanol (80 ml). Anhydrous K_2CO_3 (1g) and benzyl chloride (1 ml) were added to the suspension. The resulting mixture was heated under reflux for 5-6 h. The product was concentrated, extracted with ethyl acetate, washed with water, dried and evaporated to give a crude product (1g 86%).

Recrystallization from methanol gave a pure sample (0.92g, 80%); m.p.:174-176°C, (lit. 175-177°C). $\bar{\nu}$ max (mull): 3540 (OH), 1675 (C=O) and 1615cm^{-1} (C=C); δ:0.78 (S, 3H, 18-Me), 3.7 (t, J~8Hz, 1H, 17-H), 5.08 (s, 2H, $PhC\underline{H}_2O$), 7.37 (S, 5H, $C_6\underline{H}_5CH_2O$).

3-Benzyloxy-6-cyanomethylene-estra-1,3,5 (10)trien-17β-ol(10a)

Sodium hydride (0,4g, 50% in oil dispersion) was washed three times with dry petroleum ether (b.p. 40-60) in a three neck flask. This was flushed with nitrogen until the last traces of petroleum ether had been removed. THF (10ml) was added to the flask and cooled to 0°C. To this suspension was added diethylcyanomethylphosphonate (1g) in THF (10ml) with stirring at 0°C. After stirring for 30 min., the keto (9) (0.4g) in THF (10ml) was added and the resulting reaction mixture stirred at room temperature for 24-36h. After this period, the reaction mixture was concentrated to a small volume, poured onto ice-water and extracted with ethyl acetate (X4). The combined organic extracts were washed with water, dried and evaporated. The crude product was subjected to preparative TLC (toluene-ethyl acetate, 2:1) to give an oily product (0.25g, 60%); $\bar{\nu}$ max (film) 3475 (OH, broad), 2220 (C$\equiv$N), 1610 and 1590cm^{-1} (C=C); δ 0.75 (S,3H, 18-Me), 3.75 (t, J~8Hz) 1H, 17-H), 5.12 (S, 2H, $PhC\underline{H}_2O$), 5.72 (S, 1H, C$\underline{H}$CN), 6.2 (S, C$\underline{H}$CN), 7.05 (m. 1H, 2-H), 7.2 (d, J~ 3Hz, 1H, 4-H), 7.45 (S, 5H, $C_6\underline{H}_5O$); (Found:M$^+$, 399.2188m, $C_{27}H_{29}O_2N$ requires 399.2198).

The oily product (0.15g) was acetylated by stirring in pyridine (5ml) and acetic anhydride (1ml) overnight to give the 17β-acetoxy derivative (10b). Recrystallization from ethanol gave analytically pure sample (0.15g, 90%), mp. 134-135°C and 148-150°C; $\bar{\nu}$ max (film) 2220 (C$\equiv$N), 1730 (C=0, 17-OAc), 1625 (C=C), 1600 and 1580cm^{-1} (C=C)δ:0.85(S,3H, 18-Me), 2.1 (s, 3H, 17-OAc), 4.8 (t, J ~ 8Hz, 1H, 17-H), 5.15(S, 2H, $PhCH_2O$), 5.75 (brs, 1H, C$\underline{H}$CN), 7.45(S, 5H, $C_6H_5CH_2O$) (found: C,78.5; H, 7.1; N, 3.1% M$^+$, 441.2303. $C_{29}H_{31}O_3N$ requires C,78.88; H, 7.08; N, 3.17%; M, 441.2304).

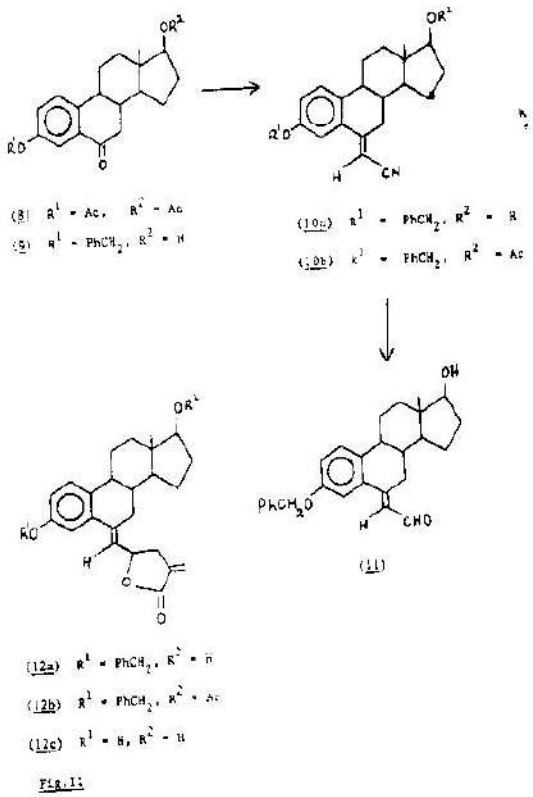


Fig. 1:

3-Benzyloxy-6-formylmethylene-estra-1,3,5(10)-trien-17β-ol (11).

The unsaturated nitrile (10) (0.45g) was taken up in dry benzene (20ml) and DIBAL (1.5ml, 25% w/v in toluene) added under nitrogen with a syringe. The reaction mixture was stirred at room temperature under nitrogen for 4h before destroying the unreacted DIBAL with methanol and the reaction mixture poured onto ice/dil. H_2SO_4. More dil. H_2SO_4 and methanol were added to dissolve solid crumbs that were formed. The mixture was extracted with diethyl ether (X4) and the combined organic extracts were washed with water, dried and evaporated. Preparative TLC of the crude product (toluene-ethyl acetate, 2:1) afforded the aldehyde (11) as oily gum (0.24g, 53%); $\bar{\nu}$ max (film) :3450 (OH, broad), 1670 (C=O, CHO) 1620 and 1600cm^{-1} (C=C); δ:10.15 (d, J~8Hz 1h, CHO), 7.42 (s,5H, $C_2H_5CH_2O$), 5.1 (S, 2H, $PhCH_2O$), 3.78 (t, J~8Hz, 1H, 17-H), 6.75(S, 3H, 18-Me) Found:M^+, 402.2192. $C_{27}H_{30}O_3$ requires M 402.2195.

(E)-5-(17β-Acetoxy-3-benzyloxy-estra-1,3,5(10)-trien-6-ylidene)-2-methylene-4-pentanolide (12)

Activated zinc (0.15g), methyl α-(bromomethyl) acrylate (0.2g) and the aldehyde (11) (0.45g) were heated under reflux in THF (100ml) under nitrogen. After about 5h, more acrylate (0.1g) was added and the reaction mixture left under reflux overnight. It was cooled, poured onto ice/dil. HCl and extracted with $CHCl_3$(X3). The combined $CHCl_3$ extracts were washed with water and brine, dried and evaporated. The crude product was subjected to preparative TLC (petroleum-ether (40-60)/Acetone, 2:1) to give an oily product (12a) Acetylation of this lactone (0.2g) in pyridine (5ml) with acetic anhydride (0.5ml) followed by further chromatographic purification gave pure lactone (12b) as an oil (0.2g, 35%). $[\alpha]_D^{20°}$ -37.5 (C, 1.5% w/v); $\bar{\nu}$ max (film):1760 (C=O,Lactone), 1735 (C=O, 17-OAc), 1600 (C=C); :7.3-6.5 (m 8H, aromatic protons), 6.5 (m, 1H, $C=CH_2$, lactone), 5.8 (d, J~9Hz =CH-CHOCO) 5.5 [m, $CH=CH_2$, (Lactone)], 5.2 (q, J~8Hz, CHOCO, Lactone),5.0 (S, 2H, $PhCH_2O$)], 4.75 (t, J 8.5Hz 17-H) Found M^+, 512.2571, $C_{33}H_{36}O_5$ requires M, 512.2563.

RESULTS

The 3-benzyloxy derivative (9) was prepared from 6-keto estradiol diacetate by reaction with benzyl chloride in the presence of K_2CO_3. This benzyloxy derivative (9) was subsequently converted to the 6-cyanomethylene derivative (10) by reaction with diethylcyanomethylphosphonate which has been found by Bose and Ramar (1968) to be quite reactive with 6-keto steroids, primarily because of the linear nature of the reagent which makes it to have low steric demands. The IR spectrum of (10) confirmed the presence of the nitrile ($\bar{\nu}$ max 2220cm^{-1}). The ^{1}HMMR Spectrum showed signals for the olefinic proton (=CHCN) at 5.72 and 6.2. These were assigned E- and Z- isomers respectively and integration suggested that E:Z ratio is approximately 4:1. The nitrile (10a) could not be recrystallized but acetylation by reaction with acetic anhydride in pyridine gave the 17 β-acetoxy derivative (10b) which was recrystallized. After recrystallization the E-isomer was obtained.

Reduction of the unsaturated nitrile (10) with di-isobutylaluminium hydride (DIBAL) in benzene, with an approach similar to that of Trofimenko (1964), followed by acid hydrolysis afforded the α, β -unsaturated aldehyde (11), presumably the E-isomer (50%). The IR showed the expected carbonyl stretching band of the α,β-unsaturated aldehyde at $\bar{\nu}$ max 1680cm^{-1}. The ^{1}HNMR Spectrum gave important signals at δ 10.15 (d, J-8Hz, CHO) and δ 6.5 (d, J~8Hz =CHCHO). Reaction of (11) with methyl α -(bromo-methyl) acrylate (prepared from CH_2N_2 and (bromomethyl) acrylic acid) and activated zinc gave the expected lactone. Preparative TLC was carried out to free the product of polymerized by-products of the acrylate. The methylene lactone (12a) thus isolated could not be recrystallized.

This was acetylated with acetic anhydride in pyridine to give the acetylated lactone (12b). The ^{1}HNMR confirmed the presence of the α-methylene lactone moiety and showed important signals at δ 6.5 (brs, $C=CH_2$), δ 5.5 (brs. $C=CH_2$) δ 5.2 (q, J~8Hz CHOCO of lactone) and δ 5.8(d, J~8Hz =CH-CHOCO).

DISCUSSIONS

The rational behind the synthesis of the α-methylene lactone (12b) was based on earlier observations. The discovery of estrogen receptor with specific binding affinity in human breast cancer provided the basis for the design. Hamacher et al (1980) noted that the use of estrogen hormones as biological carriers will have a good chance of increasing tumour cell selectivity of cytotoxic groups because of the following reasons:

(a) a high proportion of human breast cancer contain estrogen receptors.

(b) Estrogen hormone molecules show high binding affinity to estrogen receptors.

(c) The transport mechanism of carrier - receptor complexes could

result in accumulation of cytotoxic derivatives in the nucleus of the target cells which is the potential site of action, particularly alkylating agents.

Thus coupling of α -methylene lactone (the alkylating agent) at a position where presumably the binding affinity of the estrogen will not be altered drastically was conceived.

The C-6 was chosen because of its remoteness from the hydroxy groups at C-3 and C-17 which are essential for binding and the ease of functionalizing this position. The coupling of α-methylene lactone at C-6 of steroids has been attempted by Lee et al (1975) without success, presumably because of steric hindrance. It was anticipated that the extension of 6-keto function through an alkyl group could make it less sterically hindered.

The Horner-Wittig (or Edmons-Wardsworth) modification of Wittig reaction known to be more nucleophilic (Walker, 1979) was employed for this extension via the cyanomethylene derivative (10). The predominance of the E-isomer over the Z-isomer is suggestive that the E-isomer is more thermodynamically stable. The thermodynamic control arises because of the reversibility of the addition of the phosphorus stabilized carbanions to the carbonyl compound (9). DIBAL reduction of the nitrile involves its conversion to the imine derivative followed by acid hydrolysis to the aldehyde. The large vicinal spin-spin coupling constant of the aldehydic proton to the neighbouring methine proton (=CH-CHO) is not totally unexpected as similar couplings have been observed in α,β -unsaturated aldehydes (Williams and Fleming; 1980).

The Reformatsky type reaction, better known as Drielding Schimdt reaction has been shown to be versatile for conversion of aldehydes and ketones to lactones (Lee et al 1975, Hoffmann and Rabe, 1985). Thus, its application was an extension of earlier reports. However, the reaction in this case was found to lead to extensive polymerization of the acrylate which could not be easily separated from the steroidal conjugate. Hence, the 17-hydroxy lactone (12a) was acetylated using acetic anhydride in pyridine. This made it possible for the product to be separated from the by-products.

CONCLUSION:

A steroidal α -methylene lactone has been synthesized. Since the steroid moiety is an estrogen, it is hoped that the lactone (12b) will have binding affinity for estrogen-receptor when the protective groups (that is, 3-benzyloxy and 17-acetoxy) are removed. Thus the probable lactone obtained (12c) could possibly be effective against tumours located at sites that have estrogen receptors.

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