Synthesis and Structure Proof of a Vitamin D_3 Metabolite, 25(*S*),26-Dihydroxycholecalciferol

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In 1970, DeLuca and co-workers discovered a major metabolite of vitamin D_3 which exhibited weak stimulation of intestinal calcium transport and bone calcium mobilization.[1] These workers described this substance as 25,26-dihydroxycholecalciferol and its gross structure was confirmed by synthesis.[2,3] Subsequently, both C-25 epimers have been separated and tested biologically. We now describe the synthesis of pure crystalline 25(*R*),26- and 25(*S*),26-dihydroxycholecalciferol and show unequivocally that *the natural human metabolite possesses the 25S absolute configuration.* Our study necessitates the structural revision of all prior publications on "natural 25,26-dihydroxycholecalciferol,"[4-10] which was incorrectly assigned the 25*R* stereochemistry (vide infra). The assignment is based on the X-ray analysis of authentic 25(*R*),26-dihydroxycholesterol, high-performance LC comigration of the tris(trimethylsilyl) derivatives of the natural and synthetic vitamin D_3 metabolite, and lanthanide-induced CD Cotton effect measurements.

The synthesis of 25(*R*),26- and 25(*S*),26-dihydroxycholesterols was accomplished using the alkylation–reduction method of Wicha and Bal.[11] Both of the side chain precursors were accessible from the known antipodes of acid lactone **1**, readily obtained from levulinic acid.[12] (*R*)-(+)-Lactone acid (**1**), mp 88–89 °C, $[\alpha]_D$ +17° (*c* 2, H_2O), was reduced ($LiAlH_4$, THF, 60 °C) to triol **2a**, $[\alpha]_D$ + 3° (*c* 1, CH_3OH), which cleanly formed a single acetonide (acetone, TsOH, 25 °C) (Scheme I).[13] This substance was converted to the tosylate (TsCl, pyridine, O °C) which was then displaced (NaI, acetone, 50 °C) to the iodide **3a**, $[\alpha]_D$ + 2° (60% overall yield from (*R*)-(+)-**1**). In the same manner (*S*)-(−) lactone acid (**1**), mp 88–89 °C, $[\alpha]_D$ −17° (*c* 2, H_2O), was converted to triol **2b**, $[\alpha]_D$ −3°(*c* 1, CH_3OH), and hence to iodide **3b**, $[\alpha]_D$ −2°. To check the optical purities, triols **2a** and **2b** were treated with fully resolved (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride.[14,15] The bis[(+)-MTPA] esters were shown by 100-MHz NMR and HPLC analysis to have at least 99% ee.

Commercially available dehydroepiandrosterone was readily converted to the known ester **4**.[11] This substance was alkylated with iodide **3a** (lithium diisopropylamide, THF–HMPT, −40 °C)[16] to yield monoalkylated ester **5a**, mp 181–183 °C, $[\alpha]_D$ −11°, in over 80% yield (Scheme II). No dialkylated ester and little of the corresponding 20*S* alkylation product was detected. Ester **5a** was reduced ($LiAlH_4$, THF, 60 °C), esterified (TsCl, pyridine, O °C), and hydrogenolyzed ($LiAlH_4$, THF, 60 °C) to oily acetonide **6a**, $[\alpha]_D$ −21°. Exposure of **6a** to acidic methanol at 0 °C then afforded pure 25(*R*),26-dihydroxycholesterol [**7a**, mp 198–200 °C; $[\alpha]_D$ −20° (*c* 0.5, DMF)] in 55% overall yield from **4**. The corresponding 3,26-diacetate exhibited mp 154–155 °C; $[\alpha]_D$ −32°.

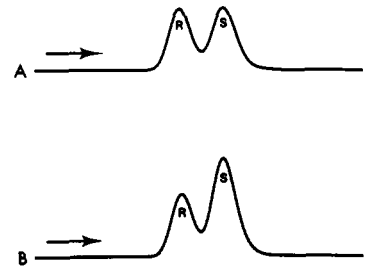

Figure 1. R = 25(*R*),26-Dihydroxycholecalciferol tris(trimethylsilyl) ether, and S = 25(*S*),26-Dihydroxycholecalciferol tris(trimethylsilyl) ether. Chromatograph, WATERS 244; eluant, 4:1 hexane–methylene chloride; flow rate, 4 mL/min, 1 recycle; column, 2x μ Porasil; detector, UV 254 nm.

The Redel group described a "25(*S*),26-dihydroxycholesterol", mp 199 °C, proven by X-ray analysis,[9] and the 3,26-diacetate derivative, mp 154 °C.[4,7] Since a discrepancy existed, we carried out an X-ray crystallographic analysis on our sample of 25-(*R*),26-dihydroxycholesterol, mp 198–200°C, and this analysis confirmed our assignment. We then requested and received the unpublished crystallographic data from the Redel group and showed that this data was also in agreement with our 25(*R*),26-dihydroxycholesterol assignment. Further communication with these workers determined that an error in drawing the completed X-ray structure led to the misassignment of the C-25 stereochemistry.[10] This error caused the misassignment at C-25 of the natural vitamin D_3 metabolite which, in fact, was derived from authentic 25(*S*),26-dihydroxycholesterol (**7b**, mp 187–189 °C).[17]

25(*S*),26-Dihydroxycholesterol (**7b**) was prepared as follows. Ester **4** was alkylated[11] with iodide **3b** to yield the monoalkylated ester **5b**, mp 176–178 °C, $[\alpha]_D$ −31°, in over 80% yield. This ester was submitted to the hydrogenolysis sequence to yield oily acetonide **6b**, $[\alpha]_D$ −46°, which was treated with acidic methanol to yield 25(*S*),26-dihydroxycholesterol [**7b**, mp 187–189°, $[\alpha]_D$ −32° (*c* 0,5, DMF)] in 61% overall yield from **4**. The corresponding 3,26-diacetate exhibited mp 120–122 °C and $[\alpha]_D$ −37°. These structures were also previously incorrectly assigned.[4,7]

The cholesterols **7a** and **7b** were straightforwardly converted into the corresponding 5,7-dienes **8a**, mp 199–201 °C, $[\alpha]_D$ −75° (*c* 0.5, DMF), and **8b**, mp 191–193 °C, $[\alpha]_D$ −97° (*c* 0.5, DMF).[18] These dienes or preferably the corresponding 25,26-acetonides were photolyzed by using a mercury lamp to the corresponding previtamins which were thermolyzed at 80 °C and deprotected with acidic methanol. High-performance LC purification and recrystallization afforded pure 25(*R*),26-dihydroxycholecalciferol [**9a**, mp 126–128 °C, $[\alpha]_D$ +88° (*c* 0.5, CH_3OH)] in 33% overall yield from **6a** and 25(*S*),26-dihydroxycholecalciferol [**9b**, mp 140–142°, $[\alpha]_D$ +91° (*c* 0.5, CH_3OH)] in 35% overall yield from **6b**.

(1) Suda, T.; DeLuca, H. F.; Schnoes, H. K.; Tanaka, Y.; Holick, M. F. *Biochemistry* **1970**, *9*, 4776.
(2) Redel, J.; Bell, P. A.; Bazely, N.; Calando, Y.; Delbarre, F.; Kodicek, E. *Steroids* **1974**, *24*, 463.
(3) Lam, H.-Y.; Schnoes, H. K.; DeLuca, H. F. *Steroids* **1975**, *25*, 247.
(4) Redel, J.; Miravet, L.; Bazely, N.; Calando, Y.; Carre, M.; Delbarre, F. *C. R. Hebd. Seances Acad. Sci. Paris, Ser. D* **1977**, *285*, 443.
(5) Queille, M. L.; Miravet, L.; Bordier, P.; Redel, J. *Biomedicine* **1978**, *28*, 237.
(6) Redel, J.; Bazely, N.; Tanaka, Y.; DeLuca, H. F. *FEBS Lett.* **1978**, *94*, 228; **1980**, *113*, 345.
(7) Koizumi, N.; Morisaki, M.; Ikekawa, N. *Tetrahedron Lett.* **1978**, 2899.
(8) Redel, J.; Bazely, N.; Mawer, E. B.; Hann, J.; Jones, F. S. *FEBS Lett.* **1979**, *106*, 162; **1980**, *113*, 345.
(9) Cesairo, M.; Guilhem, J.; Pascard, C.; Redel, J. *Tetrahedron Lett.* **1978**, 1097.
(10) Cesario, M.; Guilhem, J.; Pascard, C.; Redel, J. *Tetrahedron Lett.* **1980**, 1588.
(11) Wicha, J.; Bal, K. *J. Chem. Soc., Chem. Commun.* **1975**, 968. *J. Chem. Soc., Perkin Trans. 1* **1978**, 1282.
(12) Mori, K. *Tetrahedron* **1975**, *31*, 1381.
(13) Optical rotations were taken in 1% chloroform solution at 24 °C unless otherwise indicated. All substances were completely characterized spectrally and gave acceptable combustion analyses.
(14) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
(15) Uskokovič, M. R.; Lewis, R. L.; Partridge, J. J.; Despreaux, C. W.; Pruess, D. L. *J. Am. Chem. Soc.* **1979**, *101*, 6742.
(16) We have carried out a variety of alkylations of ester **4** and its 1α-(2-tetrahydropyranyloxy) analogue and in all cases the natural 20*R* products predominated. By this method 1α,25-dihydroxycholesterol, mp 162–164 °C, 24(*R*),25-dihydroxycholesterol, mp 200–202 °C, 1α,24(*R*),25-trihydroxycholesterol, mp 218–221 °C, 1α,25(*R*),26-trihydroxycholesterol, mp 235–238 °C, and 1α,25(*S*),26-trihydroxycholesterol, mp 197–199 °C, were prepared.
(17) The error in the C-25 stereochemistry was also independently detected by R. Barner, J. Hübscher, J. J. Daly, and P. Schönholzer (Roche, Basle).
(18) This was accomplished by sequential bromination (1,3-dibromo-5,5-dimethylhydantoin), dehydrobromination (*sec*-collidine) and deprotection (MeOH, TsOH). For an alternate 5,7-diene synthesis, see: Confalone, P. N.; Kulesha, I.; Uskokovič, M. R. *J. Org. Chem.*, in press.

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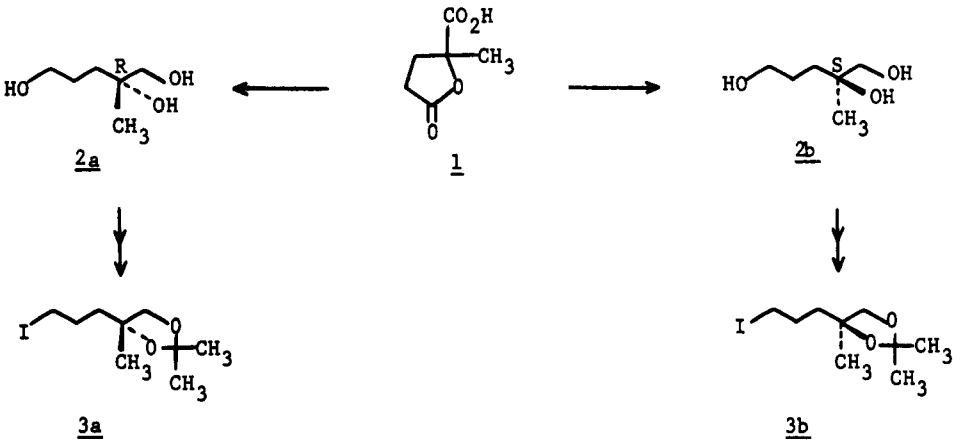
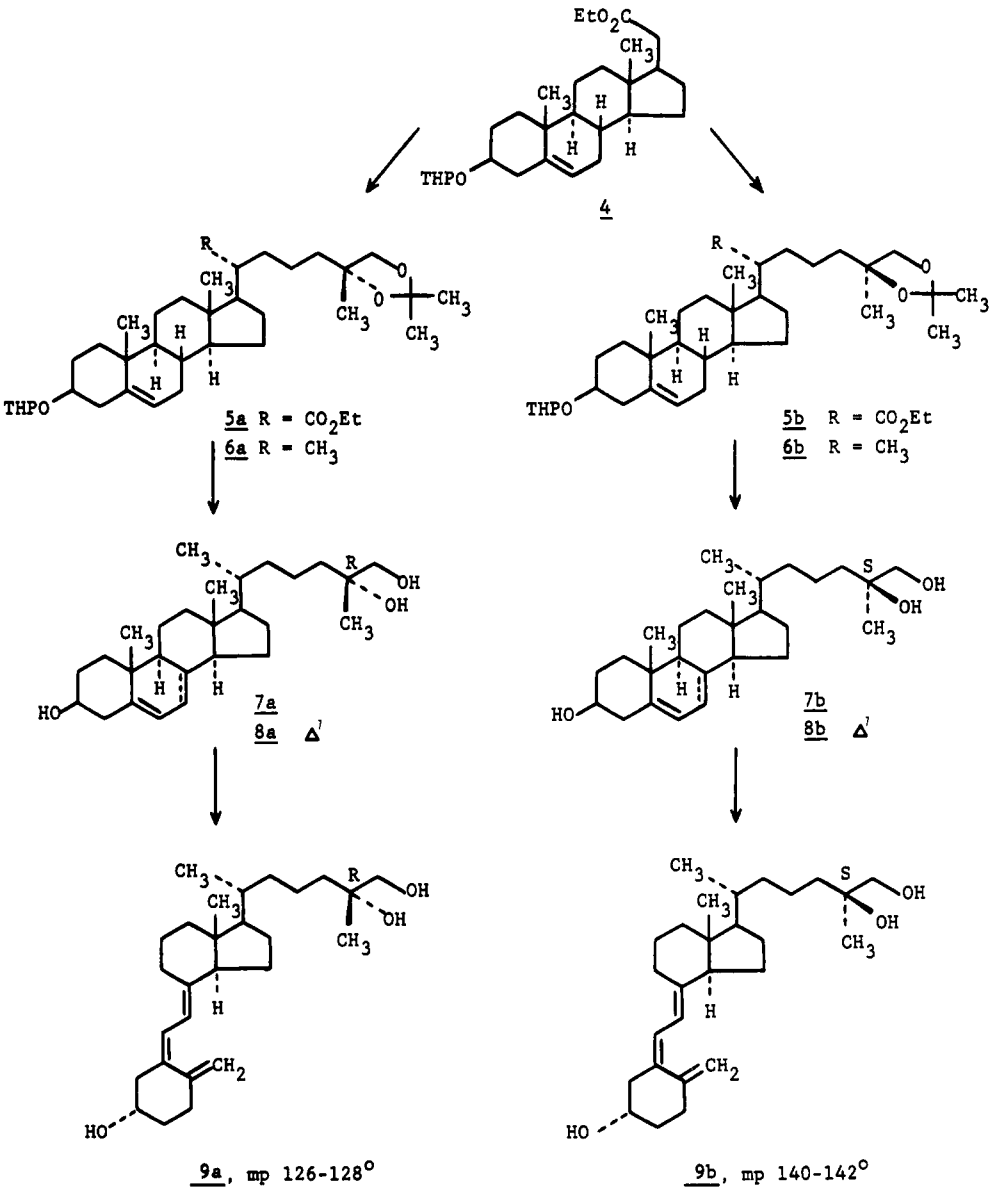
Scheme I



Scheme II



For further characterization of the 25,26-diol pairs **7** and **9**, we studied the lanthanide-induced CD Cotton effects by using our modification[19,20] of the method of Nakanishi and Dillion.[21] The Cotton effects were determined by using 1:1 complexes of $Eu(fod)_3$ and substrate (c 0.0002 M) in dry chloroform. 25-

(19) Partridge, J. J.; Toome, V.; Uskokovič, M. R. *J. Am. Chem. Soc.* **1976**, *98*, 3739.

(20) Partridge, J. J.; Shiuey, S.-J.; Baggiolini, E. G.; Hennessy, B.; Uskokovič, M. R. In "Vitamin D: Biochemical, Chemical, and Clinical Aspects Related to Calcium Metabolism"; Walter de Gruyter, Inc.: Berlin, 1977; pp 47–55.

(*R*),26-Dihydroxycholesterol (**7a**) and 25(*R*),26-dihydroxycholecalciferol (**9a**) have positive Cotton effects centered at 318 nm (ϵ +2.6 ± 0.3). Conversely, 25(*S*),26-dihydroxycholesterol (**7b**) and 25(*S*),26-dihydroxycholecalciferol (**9b**) exhibit negative Cotton effects at 318 nm (ϵ −2.6 ± 0.3). The observed Cotton effects cannot predict the absolute configuration at C-25 based on the empirical model (secondary–tertiary α-diols) of Nakanishi and Dillon.[21] *However, we have unequivocally shown that for these first examples of primary–tertiary α-diols, the R compounds have positive Cotton effects and the S compounds have negative Cotton effects at 318 nm. This may be a general phenomenon for primary–tertiary α-diols.*

To prove unequivocally the absolute configuration of the natural human 25,26-dihydroxycholecalciferol, we compared the high-performance LC elution times of the tris(trimethylsilyl) derivatives of the natural metabolite and the synthetic 25(*R*),26- and 25(*S*),26-dihydroxycholecalciferols. An approximately 1:1 mixture of the tris(trimethylsilyl) derivatives, individually made with (trimethylsilyl)imidazole (THF, 25 °C), gave the high-performance LC trace A of Figure 1. This tracing demonstrates that the 25*R* epimer is eluted before the 25*S* epimer in discrepancy with the Redel findings.[6,8] A sample of human metabolite was prepared by methylene chloride extraction of 72 mL of human serum and purification of the fraction containing the desired metabolite on a Sephadex LH-20 column. This sample was purified further by high-performance LC (μ Porasil column; 87:13 hexane–2-propanol as eluant) and then silylated. Addition of this silylated derivative[22] to the ca. 1:1 mixture of silylated synthetic epimers previously described gave the high-performance LC tracing B shown in Figure 1, which clearly shows that *the human 25,26-dihydroxycholecalciferol possesses the 25S absolute configuration.*[23]

Acknowledgment. We thank Dr. V. Toome for the circular dichroism measurements and Dr. J. P. Mallon for preparing the blood serum samples. We thank the staff of our Physical Chemistry Department for spectra and analyses.

Supplementary Material Available: Crystallographic data for **7a** and Eu(fod)$_3$-induced shift CD spectra for **7a**, **7b**, **9a**, and **9b** (8 pages). Ordering information is given on any current masthead page.

(21) Nakanishi, K.; Dillon, J. *J. Am. Chem. Soc.* **1975**, 97, 5417; Nakanishi, K.; Schooley, D. A.; Koreeda, M.; Dillon, J. *J. Chem. Soc. Chem. Commun.* **1971**, 1235. In all cases secondary–tertiary α-diols were studied.

(22) The silyl derivative of the human metabolite was identified by its characteristic ultraviolet spectrum and high-resolution mass spectrum. For the ^{13}C NMR spectrum of 25(*S*),26-dihydroxycholecalciferol, see: Williams, T. H., Greeley, D. N.; Baggiolini, E. G.; Partridge, J. J.; Shiuey, S.-J.; Uskoković, M. R. *Helv. Chim. Acta* **1980**, *63*, 1609.

(23) Since 25(*S*),26-dihydroxycholecalciferol is further metabolized to calcidiol lactone and 1α,25,26-trihydroxycholecalciferol, these substances should also possess the 25*S* absolute configuration. Portions of this work were presented at the Third IUPAC Symposium on Organic Synthesis. See: Partridge, J. J.; Shiuey, S.-J.; Chadha, N. K.; Baggiolini, E. G.; Confalone, P. N.; Kulesha, I.; Wovkulich, P.; Uskoković, M. R. "Abstracts of the Third I.U.P.A.C. Symposium on Organic Synthesis"; Madison, WI, June 15–20, 1980; p 74.

^{13}C NMR Spectra of the Uranyl Tricarbonate–Bicarbonate System

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We report the ^{13}C NMR identification of the important uranyl tricarbonate complex. This complex is of great commercial significance as the vehicle for extracting uranium from ore by solution mining and conventional carbonate leaching.[1] The uranyl tricarbonate complex is a rare example of a soluble carbonate complex and is extremely stable. A recent compilation[2] indicates that the formation constant for this complex is in the range 10^{18}–10^{23}. The NMR spectra of the uranyl tricarbonate–bicarbonate system also show the transition from slow to fast carbonate exchange between uranyl ion and bulk water in an easily accessible temperature range. The carbonate δ value in the complex is surprisingly close to the δ value of uncomplexed carbonate.

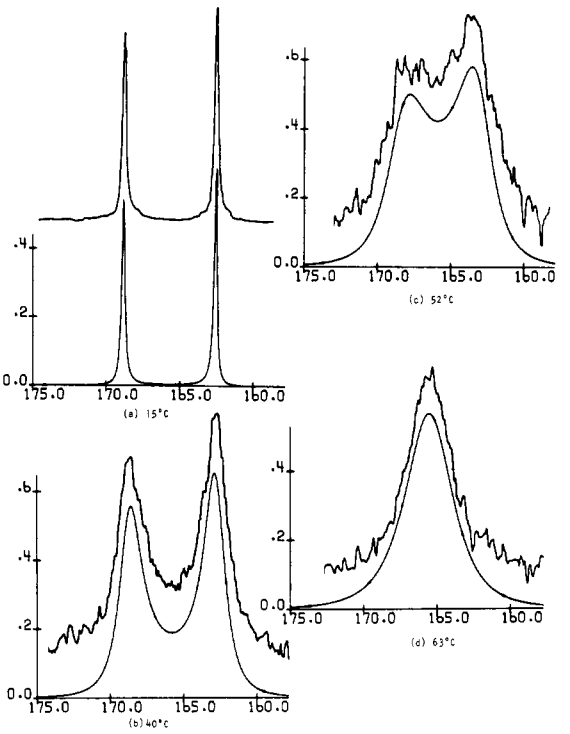

Figure 1. Experimental (above) and theoretical (below) ^{13}C NMR spectra of the uranyl tricarbonate–bicarbonate system at various temperatures, pH 9.1.

A room temperature, 15-MHz ^{13}C NMR spectrum was taken of a solution 0.0295 M in UO_2^{2+} and 0.242 M in HCO_3^- with 10% ^{13}C label at pH 8.84 in 75:25 H_2O–D_2O. If UO_2^{2+} complexes three carbonates, the carbonate concentration should be 0.0885 M in $UO_2(CO_3)_3^{4-}$ and 0.1535 M in HCO_3^-. The spectrum in fact shows two peaks. The smaller peak is at 168.86 ± 0.2 ppm, and the taller peak is at 162.31 ± 0.2 ppm.[3] In simple carbonic systems we find that uncomplexed CO_3^{2-} has a δ value of 169.6 ± 0.2 and uncomplexed HCO_3^- has a δ value of 162.1 ± 0.2, in substantial agreement with earlier reported measurements.[4] Carbonate–bicarbonate mixtures give an averaged, singlet spectrum whose spectrum δ value is weighted by the relative proportions of each. The simplest interpretation is that the taller peak at 162.31 ppm is from HCO_3^-, and the smaller peak at 168.86 ppm arises from the uranyl tricarbonate complex.[5]

Variable temperature studies offer persuasive evidence that this interpretation is correct. With increasing temperature the peaks

(1) See: Merritt, R. C. "The Extractive Metallurgy of Uranium"; U.S. Atomic Energy Commission: Washington, DC, 1971; pp 83–108.

(2) Cinnéide, S. Ó.; Scanlon, J. P.; Hynes, M. J. *J. Inorg. Nucl. Chem.* **1975**, *37*, 1013–1018.

(3) An external reference of 90% $^{13}CH_3OH$ was used whose δ value was taken as 49.3 to the left of tetramethylsilane.

(4) The δ value of CO_3^{2-} is given as 170 and that of HCO_3^- as 160 in Stothers' book (Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 304), as calculated by Stothers from the original work: Patterson, A. Jr.; Ettinger, R. *Z. Electrochem.* **1960**, *64*, 98–110.

(5) Spectra of uranyl tricarbonate complex were not mistaken for those of uncomplexed carbonate, whose δ value is similar, because in later experiments at pH 12 separate peaks could be seen for uncomplexed and complexed carbonate.

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