[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY]

The Synthesis of 20β,21-Dihydroxy-4-pregnen-3-one and 3,5-Pregnadiene-20β,21-diol*

ELIAHU CASPI

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1. 20β,21-Dihydroxy-4-pregnen-3-one was prepared from desoxycorticosterone acetate. The carbonyl at C-3 of desoxycorticosterone acetate was protected through the formation of the 3-ethylene ketal. Reduction with sodium borohydride of the carbonyl at C-20 and splitting of the ketal at C-3 gave the product.

2. Reduction of desoxycorticosterone with an excess of sodium borohydride gave a mixture of triols. The triols underwent an acid-catalyzed dehydration to yield the 20β,21-dihydroxy-3,5-pregnadiene.

For steroid metabolism studies 20β,21-dihydroxy-4-pregnen-3-one and 3,5-pregnadiene-20β,21-diol were needed. The preparation of these compounds is described in this communication.

20β,21-Dihydroxy-4-pregnen-3-one and 20α,21-dihydroxy-4-pregnen-3-one have been previously prepared.[1,2] Steiger and Reichstein[1] reduced 21-acetoxypregnenolone by the Meerwein-Ponndorf method and converted the resulting mixture of triols to the 20,21-isopropylidenes. Following oxidation of the alcohols in ring A, which led to the formation of a 4-ene-3-one grouping, the 20α,21- and 20β,21-isopropylidenes were separated by chromatography. Subsequent hydrolysis of the isopropylidenes yielded the free glycols. Julian, *et al.*[2] selectively hydroxylated the 20–21 double bond of 4,20-pregnadien-3-one with osmium tetroxide to produce primarily the 20α,21-diol. A small amount of the 20β-epimer was obtained following chromatography of the mother liquors after separation of the 20α-epimer. When the work to be reported here was completed, Norymberski and Woods[3] demonstrated that steroids containing the 4-ene-3,20-dione grouping were preferentially reduced with sodium borohydride at carbon 20 to yield the 20β-alcohols. By this method they also prepared 20β,21-dihydroxy-4-pregnen-3-one. Our approach to the synthesis was to selectively protect the carbonyl at C-3 of desoxycorticosterone acetate (formation of ethylene ketal) and reduce the carbonyl at C-20 with sodium borohydride. Removal of the ketal afforded the desired product.

Desoxycorticosterone acetate (Ia) was reacted with 2-methyl-2-ethyl-1,3-dioxolane[4] in the presence of *p*-toluenesulfonic acid to produce 21-acetoxy-5-pregnen-3,20-dione 3-ethylene ketal (II) in 82% yield. The product exhibited no selective absorption in the ultraviolet light and no indication of a conjugated carbonyl grouping could be detected in the infrared spectrum. A purple coloration, indicative of an α-ketol grouping, was obtained with blue tetrazolium (B.T.).[5,6] Reduction of II with sodium borohydride in a mixture of tetrahydrofuran and aqueous sodium hydroxide[7] gave the 20β,21-diol IIIa in 71% yield. Compound IIIa gave a negative α-ketol test. The infrared spectrum had a band at 3400 cm.$^{-1}$ indicative of a hydroxyl group; no selective absorption in the 1700–1600 cm.$^{-1}$ region was observed. Substance IIIa was converted to the diacetate IIIb, confirming the presence of two hydroxyl groups. The assignment of the "β" configuration to the hydroxyl at C-20 is based on the large positive molecular rotational increment on acetylation $\Delta M_D + 79$ (IIIa →IIIb).[8,9] The reduction of C-20 carbonyls with metal hydrides to the 20β alcohols has been previously described.[10,3] Recently Poos[11] reported the formation of both C-20 epimers upon the reduction of 17α-hydroxy-5-pregnen-3,20-dione 3-ethylene ketal with lithium aluminum hydride.

Hydrolysis of the ketal IIIa with dilute aqueous acetic acid (50% v/v) afforded in 66% yield 20β,-21-dihydroxy-4-pregnen-3-one (IVa). The diol IVa was acetylated in the usual manner to produce the diacetate IVb. The diacetate IVb was also obtained

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(1) Steiger and Reichstein, *Helv. Chim. Acta*, **21**, 171 (1938).

(2) Julian, Meyer, and Printy, *J. Am. Chem. Soc.*, **70**, 887 (1948).

(3) Norymberski and Woods, *J. Chem. Soc.*, 3426 (1955); Norymberski and Woods, *Chem. and Ind.*, 518 (1954).

(4) Dauben, Löken, and Ringold, *J. Am. Chem. Soc.*, **76**, 1359 (1954).

(5) Chen and Tewell, *Federation Proc.*, 10, 377 (1951).

(6) Chen, Wheeler, and Tewell, *J. Lab. Clin. Med.*, **42**, 749 (1953).

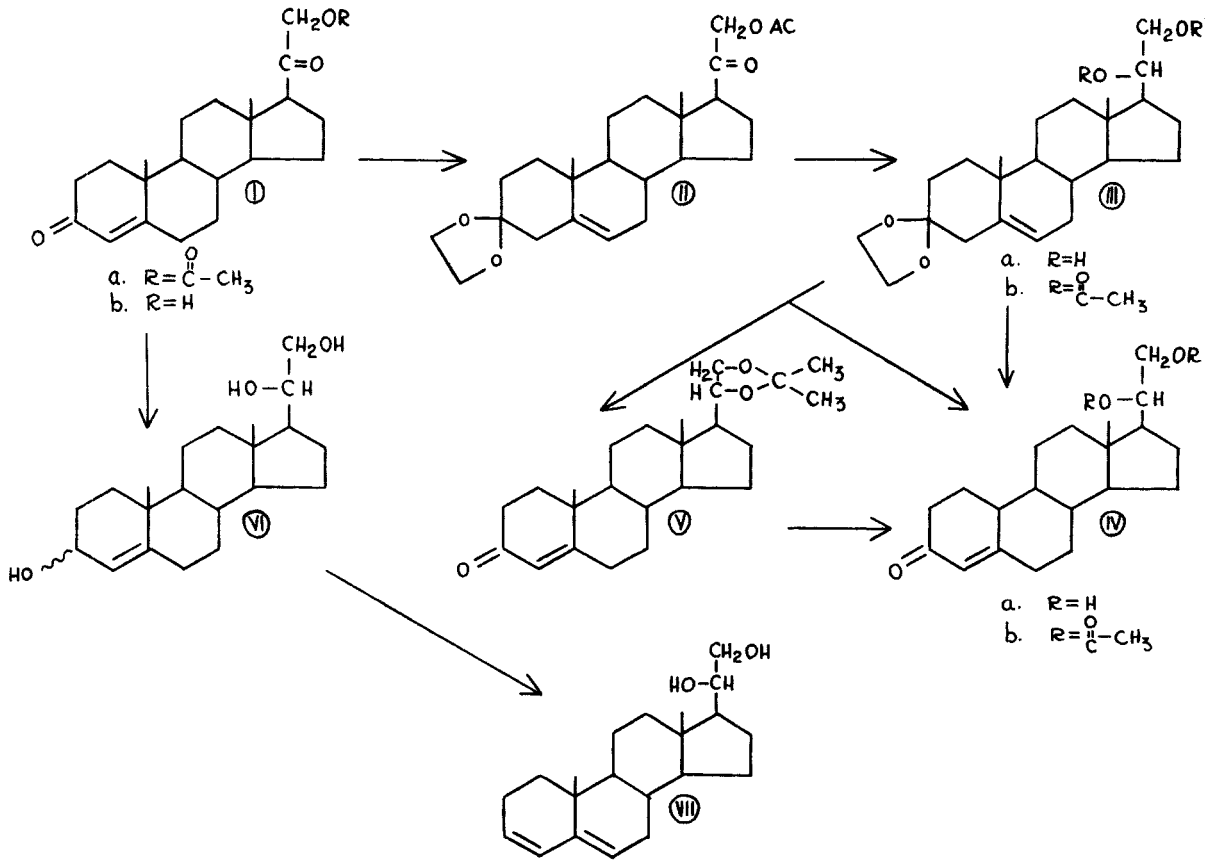
(7) Allen, Bernstein, and Littell, *J. Am. Chem. Soc.*, **76**, 6116 (1954).

(8) Fieser and Fieser, *Natural Products Related to Phenanthrene*, 3rd Edition, Reinhold Publishing Co., New York, N. Y., 1949, p. 412.

(9) Klyne in *Determination of Organic Structures by Physical Methods*, Braude and Nachod, Editors, Academic Press, Inc., New York, N. Y., 1955, p. 114.

(10) Wendler, Huang-Minlon, and Tishler, *J. Am. Chem. Soc.*, **73**, 3818 (1951); Sarrett, Feurer, and Folkers, *J. Am. Chem. Soc.*, **73**, 1777 (1951); Julian, Meyer, Karpel, and Cole, *J. Am. Chem. Soc.*, **73**, 1982 (1951); Antonucci, Bernstein, Heller, Lenhard, Littell, and Williams, *J. Org. Chem.*, **18**, 70 (1953).

(11) Poos, *J. Am. Chem. Soc.*, **77**, 4932 (1955).



from IIIb on splitting of the ketal with anhydrous acetone in the presence of *p*-toluenesulfonic acid. The increase in the molecular rotation on acetylation (IVa → IVb) ΔM_D +157° was that expected for a 20β-hydroxyl.[8,9] Furthermore the product differed from a sample of 20α,21-dihydroxy-4-pregnen-3-one obtained upon incubation of desoxycorticosterone with pig liver tissue.[12] The 20α epimer m.p. 190–193° gave a negative molecular rotational difference on acetylation ΔM_D −56°.

The diol-ethylene ketal IIIa was reacted with dry acetone and *p*-toluenesulfonic acid and gave a small amount (14%) of IVa. Chromatography of the mother liquor produced a spontaneously crystallizing residue which was presumably 20β,21-dihydroxy-4-pregnen-3-one 20,21-isopropylidene (V) (83% yield). The infrared spectrum showed the absence of hydroxyl groups as demonstrated by the lack of any selective absorption in the 3600–3300 cm.$^{-1}$ region and had bands at 1680 and 1620 cm.$^{-1}$ indicating a conjugated carbonyl. Similar instances of the formation of acetonides through the hydrolysis of steroid ethylene ketals with *p*-toluenesulfonic acid and acetone have been reported.[13,11] The crude acetonide V was hydrolyzed with sulfuric acid in aqueous methanol to produce IVa.

In Table I is a summary of the physical data determined for 20β,21-dihydroxy-4-pregnen-3-one, its diacetate and the 20β,21-acetonide. Data found in the literature are also tabulated for comparative purposes. Good agreement is observed in all cases.

For the preparation of 3,5-pregnadiene-20β,21-diol, desoxycorticosterone (Ib) was reduced with an excess of sodium borohydride at room temperature to yield what was presumably a mixture of 3α and 3β-triols VI. Woods and Norymberski[3] have reported the isolation of 4-pregnene-3β,20β,21-triol as the triacetate, when desoxycorticosterone was reduced under rigidly controlled conditions, *i.e.*, at 0°, 1 hour with 1.4–1.6 moles of sodium borohydride. The above substance was obtained as a by-product of the synthesis of 20β,21-dihydroxy-4-pregnen-3-one. When the triols VI were refluxed with *p*-toluenesulfonic acid in acetone a facile dehydration occurred to produce 3,5-pregnadiene-20β,21-diol. The 3,5-diene structure was assigned on the basis of its characteristic ultraviolet spectrum;[14] absorption maxima were observed at 228 (ε 16,500), 235 (ε 17,000), and 243 (ε 12,700) mμ. As expected for steroidal 3,5-dienes the secondary bands were separated by −7 and +8 mμ, respectively, from the

(12) Caspi, Lindberg, Hayano, Cohen, Matsuba, Rosenkrantz, and Dorfman, *Arch. Biochem. and Biophys.*, **61**, 267 (1956).

(13) Adams, Kirk, Patel, Petrov, and Stuart-Webb, *J. Chem. Soc.*, 2298 (1954).

(14) Dorfman, *Chem. Revs.*, **53**, 47 (1953).

TABLE I

COMPARISON OF PHYSICAL DATA FOUND IN THE LITERATURE FOR 20β, 21-DIHYDROXY-4-PREGNEN-3-ONE, FOR 20β, 21-DIACETATE, AND FOR 20β, 21-ACETONIDE

Compound	Physical Const.	This Report	Investigators: Steiger and Reichstein (1)	Julian, *et al.* (2)	Norymberski and Woods (3)
20β,21-Dihydroxy-4-pregnen-3-one	M.p., °C.	169–170	166–167	166–168	163–167
	$[\alpha]_D$	+94.9° (ethanol)	+92.6° (ethanol)	+98° (chloroform)	+98° (chloroform ethanol 4:1)
	$[M]_D$	+315°	+308°[a]	+326°[a]	+326°
	λ_{max} (mμ)	242	232 (hexane)		
	ε	15,600			
20β,21-Diacetoxy-4-pregnen-3-one	M.p.	153–157			155–156
	$[\alpha]_D$	+113.3° (ethanol)			+123° (acetone)
	$[M]_D$	+472°			+511°
	λ_{max} (mμ)	242			240.5
	ε	21,000			17,400
20β,21-Dihydroxy-4-pregnen-3-one 20,21-isopropylidene	M.p.	127–130	126		121–123
	$[\alpha]_D$		+91.5° (acetone)		+94° (acetone)
	$[M]_D$		+340°[a]		+350°[a]
	λ_{max} (mμ)				240.5
	ε				18,200

[a] Calculated by this author from data given.

principal one at 235 mμ. The product analyzed for $C_{21}H_{32}O_2$. The infrared spectrum showed the presence of hydroxyl groups (bands at 3725 and 3625 cm.$^{-1}$) and had two bands at 1650 (major) and 1620 cm.$^{-1}$. The frequencies of these bands were somewhat high for a 3,5-diene structure. However Bellamy[15] pointed out that the presence of another functional grouping, *i.e.*, ester, although removed from the center of conjugation caused a shift of the diene bands to high frequencies (1618 and 1578 to 1670 and 1639 cm.$^{-1}$, respectively). It appeared likely that the presence of the α-glycol group in $\Delta^{3,5}$-pregnadiene-20,21-diol also caused a similar displacement.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns hot stage and are uncorrected. Analyses were performed by Drs. G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford, England. Ultraviolet absorption spectra were determined in methanol by means of a Cary model 11 MS spectrophotometer. Optical rotations were determined in chloroform or in absolute ethanol in a 1-dm. semimicro tube. The infrared spectra were taken on a Perkin Elmer Model 12C spectrometer. With the exception of the spectrum of 3,5-pregnadiene-20β,21-diol all infrared spectra were obtained from solid films deposited on sodium chloride plates. The spectrum of 3,5-pregnadiene-20β,21-diol was obtained from a pressed potassium bromide prism.[16]

21-Acetoxy-5-pregnan-3,20-dione 3-ethylene ketal (II). A solution of 2 g. of desoxycorticosterone acetate (Ia) and 60 mg. of *p*-toluenesulfonic acid monohydrate in 30 ml. of 2-methyl-2-ethyl-1,3-dioxolane was slowly distilled until 21 ml. of distillate were obtained (75 min.). On cooling the ethylene ketal crystallized to yield 1.705 g., m.p. 194–200° The mother liquor was diluted with benzene, washed with 1 *N* sodium carbonate and water and dried over sodium sulfate. Concentration of the solution gave 149 mg. (in two crops) of less pure II, m.p. 141–145°. The infrared spectrum of the latter was essentially identical with that of the first crop. The analytical sample was crystallized three times from methanol-methylene chloride and melted at 206–208°; $[\alpha]_D^{24}$ +47° (*c*, 0.855 in chloroform), $[M]_D$ +196°; λ_{max} none; infrared ν_{max} 1750, 1732, 1240, 1232 cm.$^{-1}$.

Anal. Calc'd for $C_{25}H_{36}O_5$ (416.54): C, 72.08; H, 8.71. Found: C, 71.9; H, 8.89.

20β,21-Dihydroxy-5-pregnen-3-one 3-ethylene ketal (IIIa) A mixture of 1 g. of 21-acetoxy-5-pregnen-3,20-dione 3-ethylene ketal (II), 50 ml. of tetrahydrofuran, 1.2 g. of sodium borohydride, and 180 mg. of sodium hydroxide in 7.5 ml. water was refluxed for 24 hrs. The solvent was removed *in vacuo*, the residue was acidified with 2 *N* hydrochloric acid, and the mixture was extracted with methylene chloride. The extract was washed with water, dried, and the solvent was removed. The residue was crystallized from methylene chloride-neohexane to yield 400 mg. of a first crop, m.p. 201–204°, and 166 mg. of a second crop, m.p. 198–204°. The mother liquor was further concentrated to produce a third crop (288 mg.) of impure material which was recrystallized from ethyl acetate-neohexane to yield 81 mg., m.p. 174–184°. The analytical sample, crystallized from ethyl acetate-neohexane, melted at 216.5–217.5°, $[\alpha]_D^{26}$ −34.7° (*c*, 1.11 in chloroform); $[M]_D$ −131°; ultraviolet: λ_{max} none; infrared: ν_{max} 3400 cm.$^{-1}$; no specific absorption was observed in the 1700–1600 cm.$^{-1}$ region.

Anal. Calc'd for $C_{23}H_{36}O_4$ (376.52): C, 73.36; H, 9.64. Found: C, 72.91; H, 9.81.

20β,21-Diacetoxy-5-pregnen-3-one 3-ethylene ketal (IIIb). To a solution of 40 mg. of the diol IIIa in 1 ml. of pyridine, 0.5 ml. of acetic anhydride was added and the mixture was left for 16 hours at room temperature. The volatile components were removed *in vacuo*, water was added, and after 1 hour the mixture was extracted with ethyl acetate. The extract was washed, dried, and concentrated to a small volume. Upon addition of neohexane 39 mg. of crystalline solid, m.p. 185–191° (in two crops) was obtained. The analytical sample, crystallized from ethyl acetate-neohexane, melted at 196–199°; $[\alpha]_D^{25}$ −11.3° (*c*, 0.629 in chloroform); $[M]_D$ −52°; ultraviolet: λ_{max} none; infrared: ν_{max} 1745, 1259, 1245, 1227 cm.$^{-1}$.

Anal. Calc'd for $C_{27}H_{40}O_6$ (460.59): C, 70.40; H, 8.75. Found: C, 70.48; H, 8.76.

(15) Bellamy, *The Infrared Spectra of Complex Molecules*, Methuen & Co., London, 1954, p. 36.

(16) Rosenkrantz in *Methods of Biochemical Analysis*, David Glick, Editor, Interscience Publ. Inc., New York, N. Y., 1955, Vol. II, p. 21.

20β,21-Dihydroxy-4-pregnen-3-one (IVa). *A.* A solution of 100 mg. of IIIa in 10 ml. of 50% (v/v) aqueous acetic acid was heated 0.5 hr. on a boiling water-bath. The volatile components were removed *in vacuo*, and the residue was dissolved in ethyl acetate, washed with a saturated solution of sodium bicarbonate and water, dried and concentrated to yield 58 mg., m.p. 150–157°, of IVa. Two recrystallizations raised the m.p. to 163–165°.

B. A solution of 300 mg. of IIIa and of 30 mg. of *p*-toluenesulfonic acid monohydrate, in 30 ml. of dry acetone, was refluxed for 18 hrs. The acetone was removed *in vacuo*, and the syrup was dissolved in methylene chloride, washed with 0.1 *N* sodium hydroxide and water and dried. Upon the removal of the solvent under reduced pressure 310 mg. of a glossy syrup was obtained. The syrup was dissolved in a small volume of ethyl acetate and after 48 hours at room temperature a small amount of crystals separated. More solid separated following the addition of neohexane. The crystals were collected and washed with an ice-cold mixture of ethyl acetate-neohexane (1:1) to produce 37 mg. of IVa, m.p. 151–158°. Two recrystallizations raised the m.p. to 169–170°.

The mother liquor after the separation of IVa was concentrated under nitrogen to a syrupy residue which was dried *in vacuo* at room temperature. The syrup was dissolved in benzene and chromatographed on a 15-g. silica gel column prepared with benzene. The eluates of benzene:ethyl acetate 9:1 and 5:1 were combined to produce 213 mg. of syrup which crystallized spontaneously (m.p. 127–130°). The infrared spectrum of the product showed no selective absorption in the hydroxyl region and had bands at 1680 and 1620 cm.$^{-1}$ The compound which was presumably the 20β,21-dihydroxy-4-pregnen-3-one 20,21-isopropylidene (V) was not purified further and was hydrolyzed as follows.

A mixture of 75.3 mg. of V, 25 ml. of methanol, and 4 ml. of aqueous sulfuric acid (8% v/v), was refluxed 25 min. and left for 3 hrs. at room temperature. The solution was neutralized with sodium bicarbonate, the volatile components were removed *in vacuo*, and the residue was taken up in ethyl acetate. The ethyl acetate solution was washed with water, dried, and concentrated to a small volume. Upon addition of neohexane, 65 mg. of crude IVa was obtained, m.p. 140–150°. Two recrystallizations from the same solvents raised the m.p. to 169–170°.

The analytical sample melted at 169–170°; $[\alpha]_D^{26}$ +94.9° (*c*, 0.838 in abs. ethanol); $[M]_D$ +315°; ultraviolet λ_{max} 242 mμ, ε 15,600; infrared: ν_{max} 3590, 1675, 1623 cm.$^{-1}$

Anal. Calc'd for $C_{21}H_{32}O_3$ (332.47): C, 75.86; H, 9.70. Found: C, 75.40; H, 9.58.

20β,21-Diacetoxy-4-pregnen-3-one (IVb). *A.* A solution of 26 mg. of IIIb and of 6 mg. of *p*-toluenesulfonic acid monohydrate in 10 ml. of acetone was refluxed 18 hours. The acetone was removed *in vacuo* and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with a saturated solution of sodium bicarbonate and water, dried, and the solvent was removed. The residue was crystallized from ethyl acetate-neohexane to yield 21 mg., m.p. 128–137°, of IVb. One recrystallization raised the m.p. to 138–140°.

B. To a solution of 7 mg. of IVa in 0.25 ml. of pyridine 0.1 ml. of acetic anhydride was added and the mixture was left for 18 hours at room temperature. The mixture was worked up as described for IIIb to yield 7 mg. of solid; m.p. 137–139°. One recrystallization raised the m.p. to 139–141°.

The analytical sample melted at 153–157°; $[\alpha]_D^{27}$ +113.3° (*c*, 0.432 in abs. ethanol); $[M]_D$ +472°; ultraviolet: λ_{max} 242 mμ, ε 21,000; infrared: ν_{max} 1745, 1675, 1620, 1259, 1245, 1231 cm.$^{-1}$

Anal. Calc'd for $C_{25}H_{36}O_5$ (416.54): C, 72.08; H, 8.71. Found: C, 72.19; H, 8.83.

3,5-Pregnadiene-20β,21-diol (VII). A mixture of 290 mg. of desoxycorticosterone, 700 mg. of sodium borohydride, and 20 ml. of methanol was stirred for 18 hours at room temperature. After dilution with water the mixture was acidified and extracted with methylene chloride. The extract was washed with water, 2 *N* sodium carbonate, and water, dried, and the solvent was removed *in vacuo* to yield the crude VI. The residue was dissolved in 40 ml. of acetone and to the solution 20 mg. of *p*-toluenesulfonic acid was added. The mixture was refluxed for 6 hours and was left overnight at room temperature. The solution was neutralized with sodium bicarbonate, taken to dryness *in vacuo*, and the residue was dissolved in ethyl acetate. The extract was washed, dried, and concentrated leaving a crystalline residue (310 mg.), m.p. 80–100°.

The analytical sample was crystallized twice from methylene chloride-methanol and sublimed (100° at 0.001 mm.) melted at 124–127°; $[\alpha]_D^{24}$ −100.3° (*c*, 0.454 in chloroform); $[M]_D$ −317°; ultraviolet: λ_{max} 228, 235, 243 mμ, ε 16,500; 17,000; 12,700; infrared: ν_{max} 3725, 3625, 1650, 1620 cm.$^{-1}$

Anal. Calc'd for $C_{21}H_{32}O_2$ (316.47): C, 79.70; H, 10.19. Found: C, 79.65; H, 10.18.

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