1.

∴ ① rate of change of P:

$$V_P = k_3[ES]$$

② rate of change of ES:

$$V_{ES} = k_1\left([E_t] - [ES]\right)[S] - \left(k_2[ES] + k_3[ES]\right)$$
$$= k_1[E_t][S] - \left\{(k_1[S] + k_2 + k_3)[ES]\right\}$$

③ rate of change of S:

$$V_S = k_2[ES] - k_1\left([E_t] - [ES]\right)[S]$$
$$= (k_2 + k_1[S])[ES] - k_1[E_t][S]$$

④ rate of change of E:

$$V_E = k_2[ES] - k_1\left([E_t] - [ES]\right)[S] + k_3[ES]$$
$$= (k_2 + k_3 + k_1[S])[ES] - k_1[E_t][S]$$

Note: $[E_t]$ is the total enzyme concentration, which equals to the sum of free and substrate-bound enzyme: $[E] + [ES]$.

2. Assume the rate of changes of E, S, ES, P are:

V_E, V_S, V_{ES}, V_P

① $V_P = 150[ES]$ μM/min

② $V_{ES} = 100[S] - (100[S] + 600 + 150)[ES] = 100[S] - (100[S] + 750)[ES]$ μM/min

③ $V_S = (600 + 100[S])[ES] - 100[S]$ μM/min

④ $V_E = (750 + 100[S])[ES] - 100[S]$ μM/min

fourth-order RK method on ②:

$$\begin{cases} [ES]' = f([S], [ES]) \\ [ES]_{n+1} = [ES]_n + \frac{h}{6}(k_1 + 2k_2 + 2k_3 + k_4) \\ k_1 = f([S]_n, [ES]_n) \\ k_2 = f([S]_n + \frac{h}{2}, [ES]_n + \frac{h}{2}k_1) \\ k_3 = f([S]_n + \frac{h}{2}, [ES]_n + \frac{h}{2}k_2) \\ k_4 = f([S]_n + h, [ES]_n + hk_3) \end{cases}$$

fourth-order RK method on ③:

$$\begin{cases} [S]' = f([ES], [S]) \\ [S]_{n+1} = [S]_n + \frac{h}{6}(k_1 + 2k_2 + 2k_3 + k_4) \\ k_1 = f([ES]_n, [S]_n) \\ k_2 = f([ES]_n + \frac{h}{2}, [S]_n + \frac{h}{2}k_1) \\ k_3 = f([ES]_n + \frac{h}{2}, [S]_n + \frac{h}{2}k_2) \\ k_4 = f([ES]_n + h, [S]_n + hk_3) \end{cases}$$

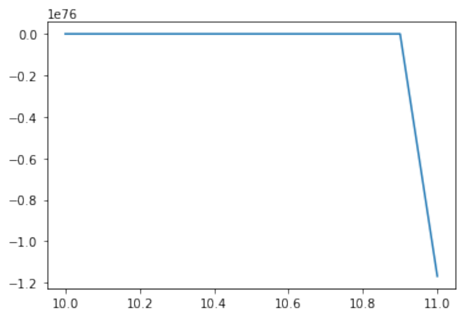
In [45]:

```
a=0
b=1
S = [10]
ES = [0]
h = 0.1   #Set the step as h
n = (b-a)/h  #Maximum number of step
def f(s,es):
    df = 100*s-(100*s+750)*es
    return df

for i in range(int(n)):
    s1 = S[i] + h
    S.append(s1)
    K1 = f(S[i], ES[i])
    K2 = f(S[i]+h/2,ES[i]+h/2*K1)
    K3 = f(S[i]+h/2,ES[i]+h/2*K2)
    K4 = f(S[i]+h, ES[i]+h*K3)
    es1 = ES[i] +h/6*(K1+2*K2+2*K3+K4)
    ES.append(es1)

result = []
for i in range(len(S)):
    result.append([S[i],ES[i]])
print(result)
import matplotlib.pyplot as plt
plt.plot(S,ES)
```

```
[[10, 0], [10.1, -22080206.375], [10.2, -872932718665334.8], [10.299999999999999, -3.530432033942479e+22], [10.399999999999999, -1.4604565466372042e+30], [10.499999999999998, -6.178854980119215e+37], [10.599999999999998, -2.6731998905042053e+45], [10.699999999999998, -1.1825105785941907e+53], [10.799999999999997, -5.347804164621207e+60], [10.899999999999997, -2.4722369610375585e+68], [10.999999999999996, -1.1681448243578875e+76]]
```

Out[45]:

```
[<matplotlib.lines.Line2D at 0x7ffe1ae1f460>]
```



when [S] changes from 10 μM → 11 μM

Rate of change of ES is shown by the slope.

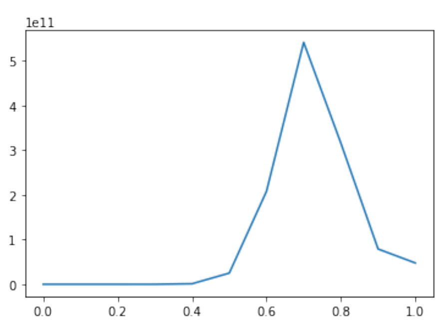
In [46]:

```
a=0
b=1
S = [10]
ES = [0]
h = 0.1   #Set the step as h
n = (b-a)/h  #Maximum number of step
def f(es,s):
    df = (600+100*s)*es-100*s
    return df

for i in range(int(n)):
    es1 = ES[i] + h
    ES.append(es1)
    K1 = f(ES[i], S[i])
    K2 = f(ES[i]+h/2,S[i]+h/2*K1)
    K3 = f(ES[i]+h/2,S[i]+h/2*K2)
    K4 = f(ES[i]+h, S[i]+h*K3)
    s1 = S[i] +h/6*(K1+2*K2+2*K3+K4)
    S.append(s1)

result = []
for i in range(len(S)):
    result.append([ES[i],S[i]])
print(result)
import matplotlib.pyplot as plt
plt.plot(ES,S)
```

```
[[0, 10], [0.1, 2336.7916666666665], [0.2, 334128.2664930556], [0.30000000000000004, 27586353.752332907], [0.4, 1208052307.3209121], [0.5, 25016749792.978893], [0.6, 207951732616.7621], [0.7, 541540970347.6514], [0.7999999999999999, 315898899381.9637], [0.8999999999999999, 78974724874.11597], [0.9999999999999999, 47713896326.98669]]
```

Out[46]:

```
[<matplotlib.lines.Line2D at 0x7ffe1af0bd30>]
```



when [ES] changes from 0 μM → 1 μM

Rate of change of S is shown by the slope.

3.
$$V = \frac{k_3[E_t][S]}{[S] + \frac{(k_2+k_3)}{k_1}} = \frac{k_3[E_t]}{1 + \frac{(k_2+k_3)}{k_1[S]}}$$ (based on Michaelis - Menten)

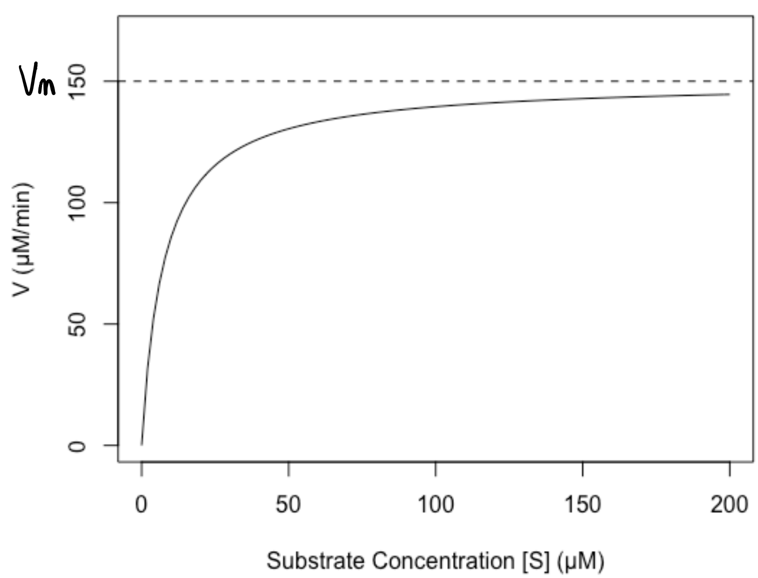
① As [S] increases, V increases.

② When $[S] \to \infty$, $\lim_{[S]\to\infty} V = \lim_{[S]\to\infty} \frac{k_3[E_t]}{1 + \frac{(k_2+k_3)}{k_1[S]}} = k_3[E_t]$

In this situation, V converge to V_{max}, which is attained when the catalytic sites on the enzyme are saturated with substrate, that is, when $[ES] = [E]_T$

plot V as a function of [S] using the values in last question:

$$V = \frac{150[S]}{[S] + \frac{15}{2}} \qquad V_{max} = \lim_{[S]\to\infty} V = k_3[E_t] = 150 \ \mu M/min$$



Appendix: the code to plot the V function:

```
V = function(S){150*S/(S+15/2)}
curve(V, from = 0, to = 200, xlab ="Substrate Concentration [S]
      ylab = "V (μM/min)", ylim = c(0,170))
abline(h=150,lty=2)
```

How to derive V:

Rate of ES formation $= k_1\left([E_t]-[ES]\right)[S]$

$[E_t]$ is the total enzyme concentration.

Rate of ES breakdown $= k_2[ES] + k_3[ES]$

At steady-state:

$$k_1([E_t]-[ES])[S] = k_2[ES] + k_3[ES]$$

$$k_1[E_t][S] - k_1[ES][S] = (k_2+k_3)[ES]$$

$$k_1[E_t][S] = \{k_1[S] + (k_2+k_3)\}[ES]$$

$$\therefore \quad [ES] = \frac{k_1[E_t][S]}{k_1[S] + (k_2+k_3)}$$

$$= \frac{[E_t][S]}{[S] + \frac{(k_2+k_3)}{k_1}} = \frac{[E_t][S]}{[S] + K_m}$$

$$\therefore V = k_3[ES] = \frac{k_3[E_t][S]}{[S] + K_m}$$