5-21-76 Hydrolysis of Dirty JE-2 DNA – by FORMIC ACID

- See p. 13 – The DNA was redissolved in TRIS, and, this morning, ~10 ml was placed into 3 beakers (i.e, the entire sample).
- add 30 ml cold (-20°C) ethanol – a copious ppt was obtained –
  observation – the DNA is cleaner, but it is still very greyish
- wash 2x with ethanol
- wash 2x with ether
- dry
- dissolve in 15 ml HCOOH – the sample was a dirty-grey color.
- let stand at room temp. for 60 min.
- centrifuge at 2.5 K rpm in PR6 for 10 — Most of the dirty material centrifuged down.
- take the clearer (not clear however) supernatant and place in Buchler tube.
- Evapomix at 40°C to dryness. The evaporation was fast at first, and then it slowed down.
- remove rest of liquid by lyoph. – it was very gummy ↓ actually flaky and amber in color.

5-24-76

- Sample was stored over weekend in the refrigerator
- add 4 ml 10% HCOOH
  Sample wouldn't dissolve completely.
- filter solids trough glass wool
  resulting solution was milky
- pump the entire 4 ml onto the reversed-phase precolumn.
- wash with 10% MeOH
- then, put in ahead of anal.. column in Micromeritics

Calculation of The Amount of Guanine Expected from Adduct Hydrolysis

Calculations on a per μg basis

← actually a base, but be conservative

Assume the adduct is a nucleoside of MW ~ 600

Aflatoxin 312
Guanosine 283
595

∴ The guanine represents ~ 1/4 of the adduct on a weight basis

$(1\ \mu g)\left(\frac{1}{4}\right) = 250$ ng of guanine released

Because of operational factors associated with hydrolysis, the conc. of base in the hydrolysate will be

$$\frac{250\ ng}{532\ \mu l} = 0.47\ ng/\mu l \approx 0.5\ ng/\mu l$$

inject 100 μl ⇒ 50ng of guanine

Student's Name

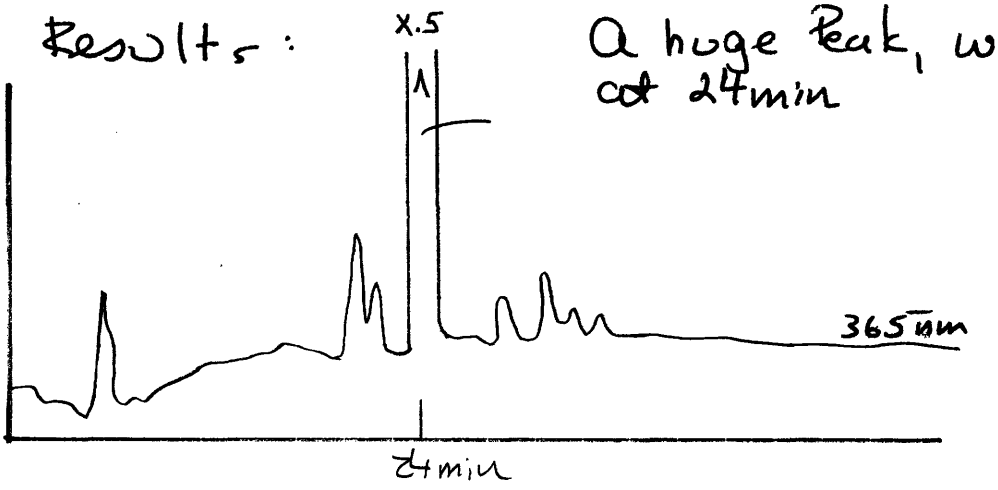
Date 5-24-76

Subject

Instructor's Name

Isolation of Peak 1 from Formic Acid Hydrolysate of JE-2 Adducted DNA

Results:



A huge Peak, was collected at 24min

- The sample was rotary-evaporated to dryness and stored in the refrigerator.

Note:
This amount of adduct was calc. by Bob Crog to be ~~~ 7.5 µg.