SYNTHESIS OF 3-INDOLYL(1-^{14}C)ACETIC, 3-INDOLYL(2-^{14}C) ACETIC AND 3-INDOLYL(1,2-$^{14}C_2$)ACETIC ACIDS

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SUMMARY

The synthesis of 3-indolylacetic acid labelled with ^{14}C in side chain carbon atoms has been effected using (1-^{14}C)glycolic acid, (1,2-$^{14}C_2$)glycolic acid and (2-^{14}C)glycolic acid in an yield of 40-60%. Some of the parameters that affect the synthesis have been studied and the results are reported in this paper.

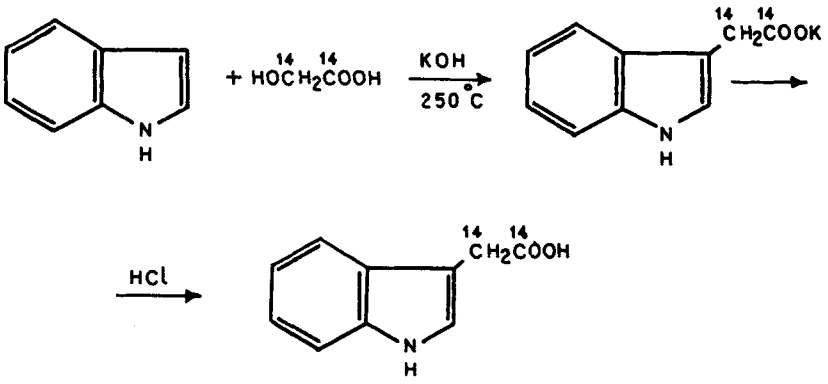
Key words:

3-Indolyl(1-^{14}C)acetic acid, 3-Indolyl(2-^{14}C)acetic acid, 3-Indolyl(1,2-$^{14}C_2$)acetic acid.

Carbon-14 labelled indolylacetic acid (IAA) has been extensively used to understand the role of IAA as a plant growth substance[1]. In order to prepare side chain ^{14}C labelled IAA, potassium(^{14}C)cyanide[2] and (^{14}C)formaldehyde[3] have been employed whereas ^{14}C labelled indole[4] has been used to prepare ring labelled IAA. This paper reports another method to obtain IAA with ^{14}C label in both or either of the side chain carbon atoms, using the appropriately ^{14}C labelled glycolic acid as the starting compound. The procedure employed is an adaptation of the non-isotopic synthesis[5] which is outlined in the following reaction scheme.

0362-4803/80/0517-0727$01.00

Received May 30, 1979



In order to scale down the synthesis from the reported 3 moles, we have employed a small stainless steel bomb and in the beginning used similar reaction conditions with respect to the ratio of glycolic acid to alkali (potassium hydroxide). We have noticed that this ratio, 1:1.3 as reported[5] was not satisfactory, since the reaction mixture at the end of the reaction contained no IAA. Considering that the composition of the stainless steel from which the bomb vessel was made may affect the results, we have tried varying ratios of glycolic acid to alkali. The experiments were carried out using 100 micromoles of (1-^{14}C)glycolic acid in each experiment and the results compared on the basis of radioactive scan of TLC on silica gel G of the reaction mixture at the end of the reaction (see figure 1). We have noticed that when the glycolic acid to alkali ratio was raised to 1:2, IAA formation was significant. Johnson & Crosby[5] have noted a significant drop in the yield of IAA when the glycolic acid solution was diluted. We have also observed a similar trend in our studies on the relationship between the yield and volume of total reaction mixture. We have thus found that 1:2 ratio of

glycolic acid to alkali and a small volume of reaction mixture which ensured a high concentration of glycolate were vital for producing IAA in good yield. We have noticed that while the yield of IAA ranged between 40-60% it was possible to recover the unreacted glycolic acid by column chromatography of the reaction mixture after precipitating out the IAA. Also in the reaction between glycolic acid and indole, the formation of other labelled side products was insignificant (see figure 1). This is an advantage for isotopic synthesis of IAA from (^{14}C)glycolic acid.

Thus, employing the conditions developed herein, we have prepared 3-indolyl(1-^{14}C)acetic acid, 3-indolyl(2-^{14}C)acetic acid and 3-indolyl(1,2-$^{14}C_2$)acetic acid from the corresponding labelled glycolic acids. The yields were in the range of 40-60%.

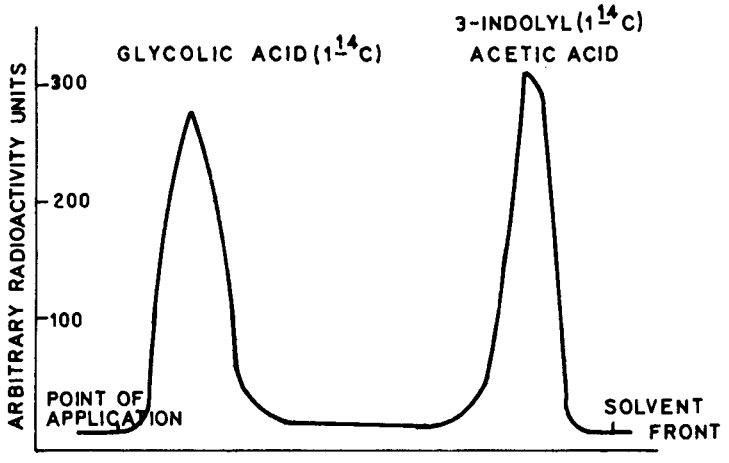


FIG. 1 RADIOSCAN OF TLC OF REACTION MIXTURE [SYNTHESIS OF 3-INDOLYL (1-^{14}C) ACETIC ACID]

EXPERIMENTAL

All reagents were of A.R. grade and distilled water was employed throughout.

(1-^{14}C)Glycolic acid was made from (^{14}C)cyanide in 90% yield,[6] (2-^{14}C)Glycolic acid and (1,2-$^{14}C_2$)glycolic acid were prepared by nitrous acid deamination of the corresponding labelled glycine in 60% yield by a procedure reported by us.[7]

Preparation of 3-Indolyl(1-^{14}C)acetic acid

(1-^{14}C)Glycolic acid solution (1 mCi, 1.5 m moles) was concentrated to dryness by rotary evaporation. Potassium hydroxide solution (0.2 ml containing 3.00 m moles KOH) was added to the glycolic acid, swirled well and carefully transferred into a small stainless steel bomb containing 2 millimoles of indole. The original flask containing glycolic acid was further washed thrice with 0.1 ml of water each time and the water washings were transferred into the reaction vessel. The bomb was tightly closed and heated in a rocking autoclave at 240-260°C for 8 hours. After cooling, the bomb was opened, the contents dissolved in 10 ml. of boiling water and then transferred to a round bottom flask. The bomb was repeatedly washed with boiling water. The combined water washings were extracted thrice with ether. The aqueous phase was rotary evaporated until the volume was 5 ml and then acdified to pH 2 by dropwise addition of dilute hydrochloric acid. The copious precipitate of 3-indolyl(1-^{14}C)acetic acid was allowed to settle at 0°-5°C in a refrigerator. After four hours the precipitate was centrifuged out and repeatedly washed with ice-cold water. The supernatant was removed and set aside for the recovery of glycolic acid. The precipitate was dried, weighed and counted. Yield 45% (120 mg, 0.45 mCi.)

An aliquot of the solution of IAA in alcohol was analysed by paper chromatography in two solvent systems:

i) n-butanol : acetic acid : water (4 : 1 : 5) and

ii) isopropanol : ammonia : water (20 : 1 : 2)

IAA was revealed by autoradiography and spray with bromocresol green. The radiochemical purity was found to be greater than 99 %.

Preparation of 3-Indolyl(1,2-$^{14}C_2$)acetic acid

The synthesis was repeated as before but using (U-^{14}C)glycolic acid. Starting from 1.2 mCi of (U-^{14}C)glycolic acid, 3-indolyl(1,2-$^{14}C_2$) acetic acid was prepared. Yield 55% (127 mg, 0.65 mCi.)

Preparation of 3-Indolyl(2-^{14}C)acetic acid

Using 0.7 mCi of (2-^{14}C)glycolic acid, 3-indolyl(2-^{14}C)acetic acid was prepared in a similar way. Yield 35% (56 mg, 0.25 mCi).

Recovery of (^{14}C) IAA from mother liquor

The mother liquors in (^{14}C) IAA preparation invariably contained another 10% of total activity as (^{14}C) IAA. In order to isolate it, a method was standardised by passing the mother liquor through a Dowex 50 x 8 (200 mesh) column (15 cm x 1 cm) and eluting with water. After the (^{14}C)glycolic acid was eluted out, the column was washed with 50% aqueous acetone. (^{14}C) IAA was eluted out separately by this procedure in a high degree of purity (see figure 2).

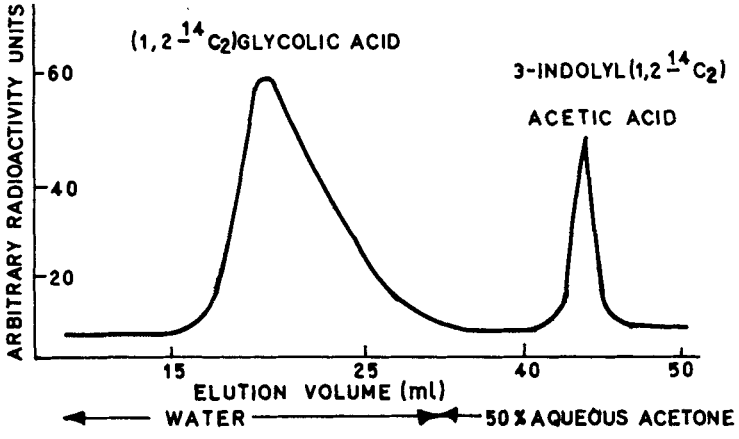


FIG. 2 SEPARATION OF 3 INDOLYL(1,2-$^{14}C_2$)ACETIC ACID &(1,2-$^{14}C_2$) GLYCOLIC ACID (CHROMATOGRAPHY ON DOWEX 50x8)

ACKNOWLEDGEMENT

The authors thank Mr. R.G. Deshpande, Head, Isotope Division for the encouragement.

REFERENCES

1. Wightman F. and Setterfield G.- Biochemistry & Physiology of Plant Growth Substances, The Runge Press, Ottawa, Canada (1968)
2. Bruce B. Stowe - Anal. Biochem. 5, 107 (1963)
3. Vandewesteringh C. and Vendstra H.- Rec. Trav. Chim. Pays Bas 77, 1115 (1958)
4. Pichat L., Audinot M. and Monnet T.- Bull. Soc. Chim. France 5, 85 (1954)
5. Johnson H.E. and Crosby D.G.-J. Org. Chem. 28, 1246 (1963)
6. Viswanathan K.V. and Ramamurthy T.V.- Paper Presented at Symp. on Ion Exchange, Bhavnagar, India (1978)
7. Viswanathan K.V. and Ramamurthy T.V.- Radiochem. Radoanal. Lett. 23, 325 (1975)