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SHORT COMMUNICATION

IMPROVED SYNTHESIS OF (RS)-2-^{14}C-ABSCISIC ACID

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(RS)-ABSCISIC acid (ABA) can be synthesized either by introduction of the ring functional groups into dehydro-β-ionylidene acetic ethyl ester’ or by side chain extension of 1-hydroxy-4-keto-α-ionone.[2] (RS)-2-^{14}C-ABA has been prepared by the first method but the yield was only 2·5 % starting with one mmole of radioactive precursor.[3] We have prepared (RS)-2-^{14}C-ABA in 17 % yield from 1-hydroxy-4-keto-a-ionone and 0·39 mmole ethyl 2-^{14}C-bromoacetate. In this method the radioactive precursor is introduced in a late step and the ratio of ABA to its *trans–trans* analog is about 1 :1.[3] The higher yields resulted from the use of techniques that minimize mechanical losses and isolation of the ABA by crystallization.

EXPERIMENTAL

Preparation of Carbethoxy-2-^{14}C-Methylene Triphenylphosphorane

To 114 mg triphenyl phosphine in 0·3 ml benzene in a 17 x 125 mm screw capped vial, 65·8 mg ethyl bromo 2-^{14}C-acetate (5 mc, nominally 12·8 mc/mmole) in 1 ml benzene was added over a 5 min period. The ampoule containing the ethyl bromo 2-^{14}C-acetate was washed with 0·2 ml benzene and the washings added to the vial.

The mixture was stored at 40° for 30 min. After overnight storage at room temp. the mixture was centrifuged, the supernatant disacrded, the crystals washed with benzene and dried at room temp. over P_2O_5 in vacuum. The salt was dissolved in 0·5 ml absolute EtOH containing a small amount of thymol blue, cooled in an ice bath and titrated to the thymol blue end point with 0·625 N NaOEt in EtOH. Solvent was removed by evaporation at room temp. in a partial vacuum and the yield used without further purification.

*Preparation of 2-^{14}C-ABA and its 2-^{14}C–*trans–trans-*isomer*

To the vial containing the yield was added 95 mg 1-hydroxy-4-keto-a-ionone,’ m.p. 11 l-l 12” from EtOH, and 0·5 ml toluene. The vial was flushed with N_2, closed and heated at 120” for 4 hr. The mixture was cooled, the solvent evaporated and 0·75 ml 10% KOH in 50% aq. MeOH added. A small teflon-coated magnet was introduced. the vial flushed with N_2 and capped. After stirring at room temp. for 14 hr 2 ml H_2O was added and the’mixture extracted 4 times with 2 ml portions of toluene.’ The toluene extracts were discarded, the aqueous phase acidified with 3N HCl, and extracted 5 times with 2 ml portions of ether. The ether extracts were combined, dried with $MgSO_4$, filtered and concentrated in a stream of N_2.

Fractionation of the 2-^{14}C-ABA Mixture

The partially crystalline residue from the ether extract was dissolved in 1 ml warm EtOAc, 3 ml toluene was added and the mixture stored overnight at 4”. The supematant was removed by centrifugation, the crystals washed with 3 ml EtOAc–toluene (1: 3, v/v) and the washings and supematant combined. The crystals were dissolved in 6 ml absolute EtOH, a sample removed for analyses, and the remainder was diluted with 54 ml benzene and stored at 4”. Specific activity was determined by liquid scintillation counting and u.v. estimation, $\epsilon_{265\,nm}$ = 19800 in 0·01 M HCl in EtOH. An analytical sample was chromatographed on

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[1] J. W. CORNFORTH, B. V. MILLBORROW and G. RYBACK, *Nature 206,715 (1965).*

[2] D. L. ROBERTS, R. A. HECKMAN, B. P. HEGE and S. A. BELLIN, *J.* Org. *Chem. 33, 3566 (1968).*

[3] J. W. CORNFORTH, R. MALLABY and G. RYBACK, *J. Chem. Soc. (c)* 1968, 1565.

TLC plates coated with H_{f254}, 0·25 mm; developing solvent EtOAc, benzene, HOAc (5: 50: 2).[4] The radioactive compounds were located with X-ray film and the proportion of each radioactive component estimated by elution and liquid scintillation counting. The mixture contained 95 % (RS)-2-^{14}*C*-ABA, 4 % *trans–trans*-2-^{14}C-(RS)-ABA-isomer amd 1 % material with R_f = 0·0. *The* total radioactivity obtained in this fraction was 0·46 mc; the specific activity before chromatography was 10·1 mc/mmole and the specific activity of the chromatographed (RS)-2-^{14}C-ABA was 10·2 mc/mmole.

The combined supernatant and washings were concentrated at reduced pressure below 40° and chromatographed. The (RS)-2-^{14}C-ABA band was eluted and crystallized with 10 mg unlabelled (RS)-ABA. The yield was 0·43 mc, specific activity 5-26 mc/mmole; chromatography showed 97% (RS)-2-^{14}C-ABA, 1 % *trans–trans*-2-^{14}C-isomer and 2% material R_f = 0·0. The *trans–trans*-2-^{14}C-(RS)-ABA-isomer band was eluted with acetone, the solvent evaporated and the residue crystallized from 0·5 ml $CHCl_3$ at −20°, m.p. 146–152°, chromatographic purity 92% (RS)-*trans–trans*-2-^{14}C-ABA-isomer, 7 % (RS)-2-^{14}C-ABA and 1 % material R_f 0·0. The yield was 0·54 mc, or 10% calculated as (RS)-*trans–trans*-2-^{14}C-ABA-isomer. The specific activity of the major component was 10·8 mc/mmole.

While none of the above preparations were chromatographically pure, they were nevertheless stored without additional purification. They can be purified further just prior to use by TLC. Precautions should be taken by minimizing exposure to light during chromatography and work-up.

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[4] B. V. MILLBORROW, in *Biochemistry and Physiology of Plant Growth Substances* (edited by F. WIGHTMAN and G. SETTERFIELDS), p. 1531, Runge Press, Toronto (1968).