p-Toluolsulfonsäure zum Sieden. Nach Zugabe von Wasser neutralisierte man mit K_2CO_3, etherte aus und erhielt 15 mg D-α-Terpienolmethylether, nach NMR-Spektrum identisch mit authentischem Material, sowie 20 mg eines Gemisches von α- und β-D-Methylglucosid-3.4-diangelicat, das nach Acetylierung mit Acetanhydrid nach DC (E–Pe 1:3) je 5 mg **6** und **7** lieferte. **6**. Farbloses Öl. IR. OAc 1750; C=CCO$_2$R 1720, 1650 cm^{-1}.

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589 \quad 578 \quad 546 \quad 436\,\text{nm}}{+76 \quad +79 \quad +89 \quad +149^\circ}(c) = 0.5)$$

Linalool-β-D-o-glucosid-3.4-diangelicat (**6**). Farbloses Öl. IR. OH 3615; C=CCO$_2$R 1730, 1650 cm^{-1}. MS. M$^+$—; —(Me)$_2$C=CHCH$_2$CH$_2$C(Me)CH=CH$_2$ 343.139 (ber. für $C_{16}H_{23}O_8$ 343.140). $[\alpha] = +27.5^\circ$ (c = 0.6).

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SYNTHESIS OF TRITIATED ABSCISIC ACID OF HIGH SPECIFIC ACTIVITY

DANIEL WALTON*, ROBERT WELLNER* and ROGER HORGAN†

*Department of Chemistry, SUNY College of Environmental Science and Forestry, Syracuse, NY 13210, U.S.A.; †Department of Botany and Microbiology, University College of Wales, Aberystwyth, U.K.

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Key Word Index—Tritiated abscisic acid; high specific radioactivity abscisic acid; basic alumina catalyzed proton exchange; synthesis.

Abstract—Stable abscisic acid (RS)-[^{3}H] was synthesized at a specific activity of 21 Ci/mmol using a basic alumina catalyzed proton exchange of 1-hydroxy-4-keto-α-ionone with T_2O followed by a Wittig reaction. Abscisic acid -[^{3}H] of specific activity 102 mCi/mmol was synthesized after carrying out a base catalyzed tritium exchange in solution.

INTRODUCTION

Abscisic acid (ABA) is a naturally occurring plant growth regulator which may be involved in the control of various plant processes. Since its discovery in 1965 [1, 2] extensive research has been carried out on its chemical, biochemical and physiological properties [3]. The absence of reports, however, concerning its cellular localization and binding may be due to the lack of a stable high sp. act. radioactive compound [4]. Both ^{14}C [5–7] and ^{3}H [8, 9] have been incorporated into ABA with a sp. act. of 6.5 Ci/mmol reported for tritiated ABA [8]. Since the tritium may begin to reexchange in aqueous solutions at pH 8 [10], this material is unsatisfactory for use in cellular localization studies.

We report the synthesis of stable ABA (RS)-[^{3}H] at a sp. act. of 21 Ci/mmol. We have used the ability of certain protons in a synthetic precursor to ABA, 1-hydroxy-4-keto-α-ionone (HKI), to exchange with those in an aqueous medium. These protons are no longer readily exchangeable after ABA is synthesized.

RESULTS

When HKI was treated with D_2O at pH 14, PMR showed that it had exchanged 9 C-borne H atoms. Six of the H were those which exchange when ABA is treated in the same way [10], while the other 3 were on the side-chain Me group (δ = 2.43). When ABA was subsequently synthesized from deuterated HKI by a Wittig reaction [11], PMR indicated that the 3 deuterons on the side-chain Me group (δ = 2.05) had not been re-exchanged although the other 6 deuterons had been. The re-exchange presumably occurred when the ABA Et ester obtained from the Wittig reaction was hydrolyzed to ABA with N NaOH. The apparent lack of re-exchange of the 3 side-chain deuterons suggested to us that stable tritiated ABA might be synthesized in the same way.

Synthesis of ABA (RS)-[^{3}H] after treatment of HKI with tritiated H_2O of sp. act. 110 mCi/mmol resulted in a product with a sp. act. of 102 mCi/mmol, indicating that 1.8 protons had been exchanged. A portion of this material was mixed with ABA-2-[^{14}C] and shaken

for 3 days at room temp. in 0.1 N NaOH. The $^3H/^{14}C$ ratio decreased only from 0.61 to 0.60 with this treatment, suggesting that the tritium was not readily re-exchangeable under basic conditions. In contrast, the 6 exchangeable protons on the ring of ABA have been reported to begin to exchange at pH 8 [10]. In an attempt to obtain ABA of higher sp. act., HKI was treated with tritiated H_2O of sp. act. 24 Ci/mmol. Several exchanges were unsuccessful apparently because of the destruction of HKI during the exchange process. Since HKI is base labile, its loss may have been due in part to an excessively high pH which was difficult to control in a reaction vol. of only 22 μl. It is also possible that part of the loss of HKI may have been caused by its contact with the high sp. act. tritiated H_2O for 2 hr.

In order to effect the exchange of acidic protons on HKI under milder and more readily controlled conditions, we investigated the use of basic Al_2O_3 as a catalyst. The exchange of acidic protons in ketosteroids [12] has been effected using a chromatographic column prepared from basic Al_2O_3 that had been equilibrated with 18 mCi/mmol tritiated H_2O. The ketosteroid was applied to the column and eluted at room temp. over a period of 20 to 60 min. We found that the exchange could be considerably improved if a mixture of basic Al_2O_3, tritiated H_2O, anisole and HKI were shaken at 120° for 20 min prior to elution of HKI from the Al_2O_3. Consequently, an exchange with carrier-free tritiated H_2O of sp. act. 60 Ci/mmol was carried out under these conditions by New England Nuclear (549 Albany St., Boston, Mass. 02118). This exchange was done successfully since TLC of the material eluted from the Al_2O_3 showed very little destruction of HKI which contained *ca* 1.25 Ci of ^3H.

The ABA-(RS)[^3H] synthesized from HKI-[^3H] [11] was purified by TLC in two solvent systems. The 2-*trans* isomer of ABA which is synthesized in quantities *ca* equal to those of ABA is separated from ABA by these procedures. Unlike ABA, much of the 2-*trans* ABA was lost during purification, apparently due to its conversion to other products. When a sample of the ABA obtained from the second TLC plate was methylated and subjected to GLC, the ABA peak appeared to have a small shoulder. Therefore, the ABA-[^3H] was further purified by HPLC which indicated that 93% of the radioactivity was associated with ABA and 4% with its 2-*trans* isomer. GLC of the HPLC-purified ABA gave a single peak with a R_t identical to that of ABA. The sp. act. of this material was determined to be 21 Ci/mmol by GLC and liquid scintillation counting.

Samples of the ABA-[^3H] purified by HPLC were made up in 50% aq. MeOH containing 0.5 M NH_4OH and incubated for 3 days at ambient temp. This treatment resulted in no measurable re-exchange of tritium, as evidenced by loss of radioactivity when the solvents were removed, although the method used was sufficiently sensitive to have detected a 0.5% loss.

DISCUSSION

We have described methods which may be used to synthesize ABA (RS)-[^3H] of either intermediate or high sp. act. The procedures involve the base-catalyzed exchange of acidic protons on the side-chain of HKI with tritiated H_2O. The subsequent conversion of HKI to ABA greatly reduces the acidity of these protons so that a 3-day treatment of ABA-[^3H] with an aq. soln containing 0.5 M NH_4OH does not result in measurable re-exchange of tritium. The exchange with tritiated H_2O of relatively low sp. act. can be carried out in soln if the pH can be controlled. If high sp. act. H_2O is used for exchange, with a concomitant small solution vol., basic Al_2O_3 can be used to catalyze the exchange. Although the extent of exchange is less on basic Al_2O_3 than in soln, the use of the Al_2O_3 enabled us to carry out the exchange using 18 μl of carrier-free tritiated H_2O (sp. act. 60 Ci/mmol) with little loss of base-labile HKI. The 21 Ci/mmol ABA-[^3H] appears to be reasonably stable since no breakdown as detected by HPLC occurred when 5 mCi were stored in 100 ml of C_6H_6–EtOH (9:1) for 8 months. We infer from our expts with D_2O that the bulk of the tritium is attached to the side-chain Me group of ABA.

Although the exchange of HKI with high sp. act. tritiated H_2O using basic Al_2O_3 as a catalyst was successful with regard to yield, we obtained only a 1% yield in the subsequent synthesis of ABA. In preliminary expts using low sp. act. tritiated HKI, we had usually obtained yields of ABA of from 6–15%. These yields were comparable to those reported for the small-scale synthesis of ABA-2-[^{14}C] [6], although we had scaled down this synthesis by a further order of magnitude. Occasionally, however, we obtained yields of only 0.5–1% for no apparent reason. We do not know whether our low yield of the high sp. act. ABA was due to the difficulties previously encountered or were caused in part by the large amount of radioactivity present during the synthesis. In spite of the low yield of high sp. act. ABA-[^3H], we believe that the techniques described can be used for the preparation of stable ABA-[^3H] which may be useful for cellular localization and binding studies.

EXPERIMENTAL

Solution exchange of HKI. A solution of 0.2 ml dioxane and 0.20 ml THO (sp. act. 110 mCi/mmol) containing 50 mg HKI and *ca* 10 mg Na was allowed to stand at room temp. for 2 hr. The mixture was then added to Et_2O (10 ml) and dried for 18 hr over molecular sieves. D_2O exchange was similar to that described for THO except that the soln contained 100 mg HKI, 1 ml D_2O and *ca* 20 mg Na.

Basic Al_2O_3 catalyzed exchange of HKI. Carrier-free T_2O (18 μl) (sp. act. 60 Ci/mmol) were added to a flask containing 350 mg of W-200 basic Al_2O_3 (M. Woelm), 0.32 ml anisole (dist. over basic Al_2O_3) and 8 mg HKI. The mixture was shaken at 120° for 20 min. The HKI was eluted from the Al_2O_3 with 100 ml pentane–THF (9:1), and the soln filtered and taken to dryness *in vacuo* at 30°. The residue was twice taken up in 20 ml EtOH and evaporated to dryness, and then taken up in EtOH, streaked on a 20 × 20 cm, 0.5 mm thick Si gel GF_{254} TLC plate and developed with pentane–*iso*PrOH (93:7). The purified HKI contained *ca* 1.25 Ci of ^3H.

Synthesis of ABA(RS)-[^3H]. ABA was synthesized essentially according to 'method B' of ref. [11]. A mixture of 2.5 mg HKI and 4.5 mg carbethoxymethylene triphenylphosphorane was heated at 170° for 45 min in the absence of solvent. The mixture of esters was saponified for 2.5 hr with 50% aq. MeOH containing 1.2 M KOH, diluted with H_2O and extracted with Et_2O for 2 hr. The aq. soln was acidified to *ca* pH 2 and extracted with Et_2O for 5 hr. The extract was dried over molecular sieves and the solvent removed.

TLC purification of ABA. Carried out on 20 × 20 cm, 0.5 mm thick Si gel GF_{254} plates in H_2O-satd $CHCl_3$–HOAc (99:1) followed by $CHCl_3$–MeOH–H_2O (75:22:3).

HPLC. Carried out using a 3 × 250 mm column packed with

Spherisorb 5 μm ODS. Elution was isocratic with either H_2O–MeOH (9:1) or H_2O–MeOH (7:3) containing 10^{-3} N HCl, flow rate 1 ml min^{-1} at 155 kg cm^2.

GLC. Carried out on ABA Me ester with an instrument equipped with an electron-capture detector and a 130 × 0.2 cm glass column packed with 3% DC-200 on Gas-Chrom Q. The carrier was Ar–CH_4 (19:1), flow rate 40 ml min^{-1}, oven temp. 165° and detector temp. 186°.

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NORBOTRYAL ACETATE, A NOR-SESQUITERPENOID ALDEHYDE FROM *BOTRYTIS CINEREA*

OLGA CUEVAS and JAMES R. HANSON
School of Molecular Sciences, University of Sussex, Brighton, Sussex BN1 9QJ, U.K.

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Key Word Index—*Botrytis cinerea*; Hyphomycetes; sesquiterpenoid; nor-sesquiterpenoid; botrydial; ^{13}C NMR.

In the course of biosynthetic studies [1] on the metabolites of the plant pathogen, *Botrytis cinerea*, we isolated an unstable oily acetoxy-aldehyde, $C_{16}H_{24}O_3$. The IR [ν_{max} 2740, 1670, 1625 (C=C.CHO) and 1735, 1235 (CH_3.CO.O) cm^{-1}] and NMR [δ 10.0 (1H, *s*) and 2.00 (3H, *s*)] revealed the presence of the aldehyde and acetate groupings. Oxidation with CrO_3 and methylation of the product with CH_2N_2 gave a monomethyl ester which was again an unstable oil. The NMR data (see Table 1) can be interpreted in terms of structure (1) for the compound which we have named norbotryal acetate. The fully substituted nature of the $\alpha\beta$-unsaturated aldehyde was confirmed by the UV [λ_{max} 253 nm (calc. 254 nm), ε 10300] and by the singlet character of the olefinic ^{13}C resonances. PMR decoupling experiments established the presence of a CH_2.CH(OAc).CH— and two CH.Me groups. The location of the other carbon atoms in two tertiary methyl groups, a methylene, a quaternary carbon atom and an acetoxyl group, was clear from the ^1H and ^{13}C NMR spectra.

The co-occurrence of this compound with botrydial (2) [2] led to the proposed structure (1). This relationship

Table 1. The NMR spectra of norbotryal acetate

Position	^{13}C resonance*	^1H resonance
1	135.8	
2	32.5	2.85 (*m*)
3	37.5	1.38 and 2.14 (*m*)
4	70.2	4.75 (octet J = 3, 9, 10 Hz)
5	56.0	2.54 (*q* J = 2 and 9 Hz)†
6	40.9	
7	49.3	1.8 (*m*)
8	29.1	3.2 (*m*)†
9	169.0	
10	190.4	10.0 (*s*)
11	21.9	1.12 (*d*, J = 6 Hz)
12	28.3	1.25 (*s*)
13	20.6	0.75 (*s*)
14	24.2	1.28 (*d*, J = 7 Hz)
C=O	170.1	
Me	21.3	2.00 (*s*)

(determined in $CDCl_3$; values in ppm from TMS)
* Assigned by comparison with botrydial and derivatives.
† These signals showed a long-range coupling.