MICROSYNTHESIS OF ^{3}H-STERCULIC ACID WITH HIGH SPECIFIC ACTIVITY

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SUMMARY

Copper-catalysed addition of ethyl diazoacetate to methyl stearolate (20 mmol scale) and subsequent saponification gave 9,10-carboxymethylene,9-octadecenoic acid in 67% yield. The latter was decarbonylated by treatment with $HClO_4/Ac_2O$ *in the cold (76 µmol scale) to give cyclopropenium perchlorate, which was reduced with* $NaBH_4$*. After removal of non-acidic by-products, sterculic acid labelled with* ^3H *in the cyclopropene ring was obtained in 9% yield. Its specific activity was 140 mCi/mmol, the radiochemical purity was 90%.*

For biochemical studies on the inhibition mechanism of the 9-desaturase enzyme system$^{1)}$, labelled sterculic acid (9,10-methano-9-octadecenoic acid) with a specific activity of 100 mCi/mmol was required. Syntheses of ^{14}C-labelled cyclopropenoid acids, recently described$^{2,3)}$, were inadequate for our purpose. Labelling at the required level of specific activity limited our possibilities to the application of ^{3}H, scaling the reaction down to a 0.1 mmol level and introducing the label at the latest possible stage. Starting from methyl stearolate, introduction of ^{3}H by reduction of the intermediate cyclopropenium ion with NAB^3H_4 seemed to be the only approach:

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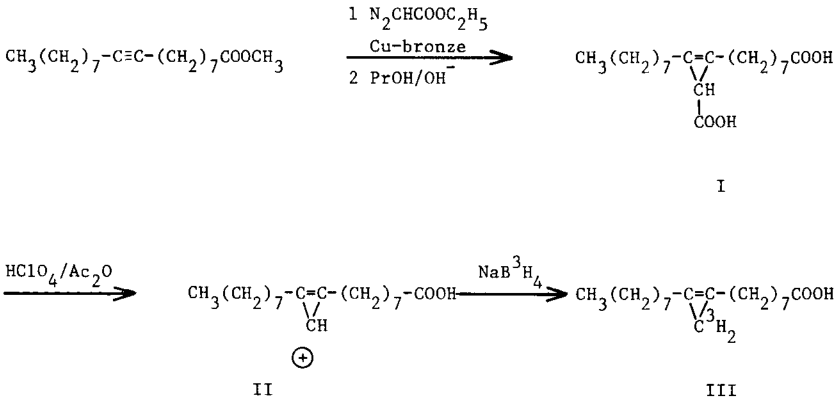

Copper-catalysed addition of ethyl diazoacetate to methyl stearolate was performed according to Gensler et al[4)] on a 20 mmol scale (67% yield). The product was purified by column chromatography and saponified with 10% propanolic NaOH[3,5)]. Since the diacid was the last intermediate that could be isolated and handled in pure form, the subsequent reaction had to be scaled down to the 100 µmol range. Of various possibilities described in the literature, only the $HClO_4/Ac_2O$ method of Pawlowski et al[3,5)] was successful on this scale. The cyclopropenium perchlorate was obtained in satisfactory yield and was subsequently reduced with borohydride. Whereas usually a 5-50-fold molar excess of $NaBH_4$ is applied, we succeeded in getting an 11.6% yield of labelled sterculic acid with a slightly less than equimolar amount of NaB^3H_4. In solvents like dimethylsulphoxide/pyridine (which dissolves both the metal hydride and the cyclopropenium salt[3,5)]) the only product was found to be stearolic acid. According to Gensler et al.[6)] this may be due to decomposition of an intermediate addition product of the cyclopropenium ion and the cyclopropene compound, which will obviously be favoured by an excess of the cyclopropenium ion in a homogeneous medium. Under our conditions the final product contained also about 30% non-labelled stearolic acid, formed during the reduction step. Separation from the product was not pursued, since its presence did not hamper the biochemical studies. The main radioactive impurities were shown to be non-acidic and could easily be removed by extraction of the acids from the light petroleum solution with aqueous NaOH, acidification, and extraction with ether. The radiochemical purity of the ^{3}H-sterculic acid was 90% by TLC and GLC, the main radioactive contaminants possibly being oxo-and/or hydroxy acids.

EXPERIMENTAL

Preparation of (9,10-methano-^{3}H)-sterculic acid (III)

In a glass-stoppered reaction tube, a mixture of 76 μmol $HClO_4$ (13 mg) and 130 μl acetic anhydride (both analytical grade ex Merck) was added at 0°C to 76 μmol of chromatographically pure 9,10-carboxymethano-9-octadecenoic acid (I). The latter had been prepared according to Gensler et al$^{4)}$. The brown reaction mixture was kept at 0°C for 30 min; unreacted perchloric acid and acetic anhydride were removed from the viscous cyclopropenium perchlorate by shaking twice with an ice-cold mixture of 100 μl anhydrous chloroform and 800 μl light petroleum.A nitrogen atmosphere was maintained during the whole procedure.

Into a 10 ml three-necked round-bottomed flask provided with a nitrogen inlet and outlet tube and a serum cap at the central neck, 25 mCi $NaB^{3}H_4$ (ex Radiochemical Centre, Amersham, England) was introduced and 1 ml light petroleum added with a syringe under magnetic stirring in a nitrogen atmosphere. The ampoule was rinsed with 75 μl saturated methanolic NaOH and the rinse also injected. The flask was then cooled to -40°C and the cyclopropenium perchlorate introduced dropwise with a syringe; 100 μl MeOH was used to complete this transfer. The mixture was left to reach 0°C in about 20 min. Then 500 μl 0.5 HCl was added and stirring continued for another 30 min. Evolving tritium gas was transported in a gentle stream of nitrogen, which was maintained throughout the reduction reaction, to a tube with PtO_2 at 130°C. The $^{3}H_2$ was converted into $^{3}H_2O$ and trapped in molecular sieve pellets.

The reaction mixture was diluted with water and extracted three times with light petroleum. GLC showed that 8.96 μmol ^{3}H-sterculic acid was formed (2.63 mg; 11.6% of theory). The crude product contained 2.14 mCi ^{3}H. The pooled organic layers were extracted twice with 2 ml 0.1 N NaOH to isolate the acid material.

The aqueous layers were acidified and extracted twice with ether; the ethereal solution was dried over Na_2SO_4. In this way 2.05 mg labelled sterculic acid (9% yield from diacid II, specific activity 140 mCi/mmol) was obtained with a satisfactory radiochemical purity (90%).

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