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APPLICATION OF THE VILSMEIER FORMYLATION IN THE SYNTHESIS OF 11-^{13}C LABELLED IRIDOIDS.

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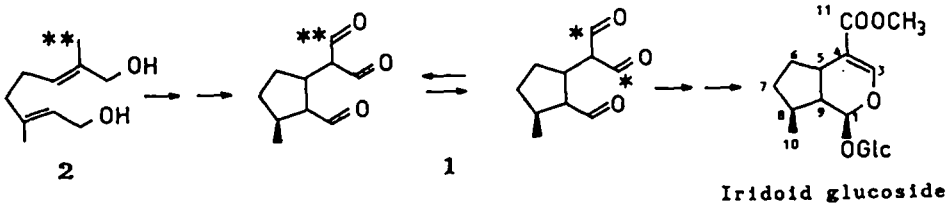
Abstract - The Vilsmeier reaction was utilized for the introduction of C-11 into iridoid glucosides. Aucubin hexaacetate (3a), 6,10-dideoxy aucubin tetraacetate (6a) and 8(S)-6,10-dideoxy-7,8-dihydro aucubin tetraacetate (7a) were used as substrates for the reaction. 6a and 7a were prepared by catalytic transfer hydrogenation of 3a with formic acid and Pd/C. The Vilsmeier reaction conditions were optimized with regard to the economic use of [^{13}CHO]-DMF in the synthesis of [11-^{13}C]-iridotrialglucoside (9). Remarkably, 5 % of the label in the product turned out to be situated at C-3.

INTRODUCTION.

The biosynthesis of iridoid glucosides has been studied in some detail[1,2] by feeding experiments with labelled precursors. A problem of major interest has been the randomization of C-3 and C-11 which takes place during the biosynthesis of many iridoids. It has been suggested[3] that randomization could take place through an intermediate such as iridotrial (1).

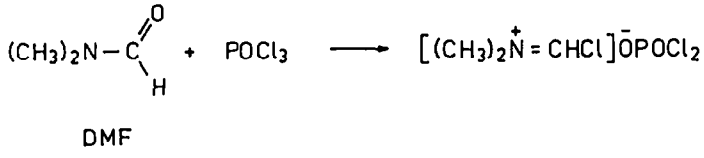


2 1 Iridoid glucosides

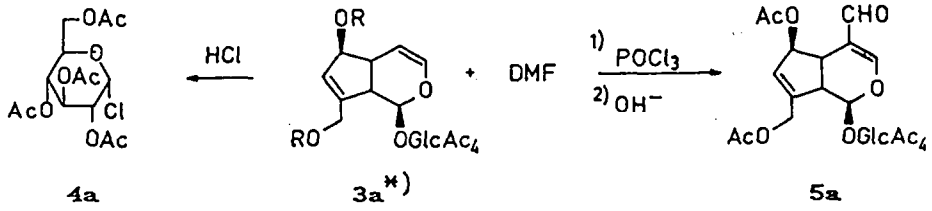
Inouye *et al.*[3] have used ^{13}C-labeled acyclic monoterpenes, such as [9-^{13}C]-10-hydroxygeraniol 2, in the study of biosynthesis involving randomization of C-3 and C-11. This paper describes a synthesis of [11-^{13}C]-labelled iridoids with an intact iridoid skeleton for similar applications.

RESULTS AND DISCUSSION.

Iridoids lacking C-11, such as aucubin (3) are readily available in useful amounts from many plant sources. The 4-position in these iridoids is activated toward electrophilic attack and thus the synthesis of an 11-^{13}C-iridoid using such an iridoid as a substrate and a ^{13}C-labelled one-carbon electrophile might be a possibility. The Vilsmeier reaction, an aldehyde synthesis employing an electrophilic formylating agent derived from formamide and phosphorous oxychloride, appeared a reasonable choice. Vilsmeier formylations are commonly performed with activated aromatic substrates (phenols, anisols etc.) but also less activated substrates such as limonene,[4] steroid enol ethers[5] and dihydropyrane can be formylated (thus a 70 % yield has been reported[6] for the latter). The presence of a dihydropyrane moiety in iridoids of the aucubin type suggests that these compunds too might be effective substrates in a Vilsmeier formylation. As 3 can be isolated in large amounts from *Aucuba japonica* (2,5 % of the fresh weight), 3a was chosen as substrate in our first experiments.



DMF



4a 3a*) 5a

*)Postscript "a" in compound numbers means the fully acetylated derivative.

Employing the reaction conditions reported for the formylation of dihydropyrane, we obtained 5a in a yield of only 14 % (Table 1, entry 1), despite a large excess of the Vilsmeier reagent. The reaction mixture contained not only unconverted 3a (60 %), but also α-D-glucopyranosyl chloride tetraacetate (4a) (15%), indicating that extensive cleavage of the glycosidic bond had taken place during the reaction. Since the major purpose of investigating the reaction was the possibility of introducing a ^{13}C label into the iridoids by means of DMF, the utilized part of the DMF was of particular interest. In the first experiments this utilization amounted to an unacceptable 0.2 %. A possible explanation might be that the solvent (1,1,2-trichloro-ethylene) gave rise to a two-phased reaction mixture. Thus an experiment using no solvent (entry 4) improved the yield of 5a rather drastically. However, as DMF was used in large excess, the utilized part was still as low as 2.1 %. Unfortunately it seemed impossible to reduce the amount of DMF under these conditions, as even the large excess of DMF which had been used was unable to dissolve the substrate completely, underlining the demand for a suitable solvent. Two experiments were performed in other solvents without succes

Table 1. Optimization of the synthesis of 5a.

entry	3a (mmol)	DMF (mmol)	$POCl_3$ (mmol)	Solvent	(ml)	Temp (°C)	time (h)	Yields 5a,%	4a,%	Utilized DMF (%)
1	0.17	13.0	6.6	TCE *)	7	88	2	14	15 **)	0.2
2	0.17	13.0	6.6	TCE	7	60	96	15	38 **)	0.3
3	0.17	3.3	1.65	TCE	3.5	60	24	<5	-	-
4	0.17	3.3	1.65	-	-	50	16	40	15	2.1
5	0.17	3.3	1.65	NO_2CH_3	1	50	16	5	5	0.3
6	0.17	3.3	1.65	DMEU***)	1	50	16	-	10	-
7	0.17	3.3	1.65	CH_2Cl_2	2.5	40	19x24	60	20	3.1
8	2.34	3.25	2.75	CH_2Cl_2	4	42	60	21	5	15
9	2.67	52	26	CH_2Cl_2	8	42	72	63	- **)	3.2

*) TCE = 1,1,2-trichloro-ethylene.

) Isolated yield. Other yields were estimated by NMR; e.g. in entry 1, the amount of **4a was determined only by NMR, in entry 9 it was not determined at all.

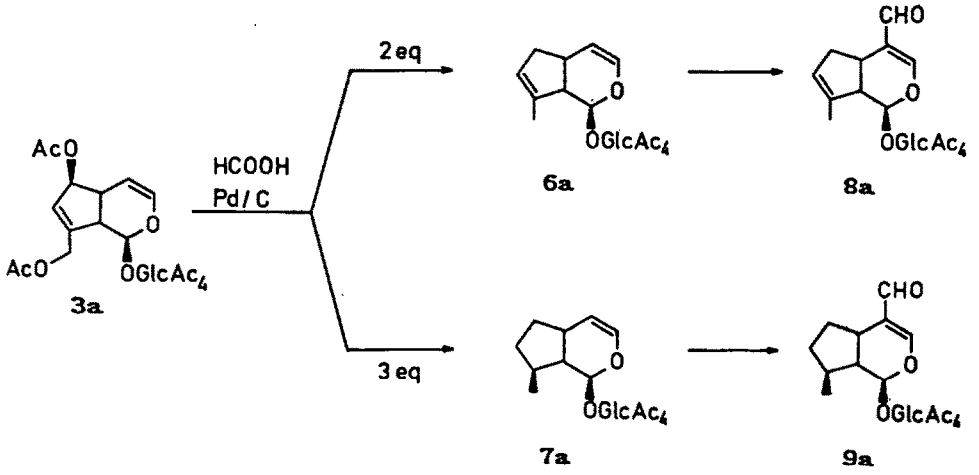
***) DMEU, 1,3-Dimethyl-2-imidazolidinone, a dipolar aprotic solvent (Merck).

(entry 5 and 6). Finally an experiment performed in dichloromethane provided the desired one-phased reaction mixture and an acceptable yield (entry 7). In order to suppress the formation of **4a**, which presumably was produced by attack of hydrogen chloride formed in the reaction, a 4 Å molecular sieve was added to the reaction mixture as a scavenger of HCl. In a second experiment with the same solvent, the amount of Vilsmeier reagent was reduced to an almost equimolar amount of the substrate (entry 8). In order to avoid (partial) saponification of the product, a saturated solution of $NaHCO_3$ was used for the work-up instead of NaOH. The yield of 5a was a moderate 21 %, but on the other hand the utilized part of the DMF this time amounted to 15 %. A final experiment was run in order to optimize the yield of 5a, using a large excess of the Vilsmeier reagent and the improved experimental conditions (entry 9). The progress of the reaction was monitored by working up small samples which were analysed by TLC. After 3 days, work up of the reaction mixture gave 5a in a yield of 63 %.

In order to test other iridoid substrates in the Vilsmeier reaction, we synthesized the two aucubin derivatives 6a and 7a. The synthesis was initially planned in analogy with that reported for deoxyloganin.[7] Thus 3 or 3a by hydrogenolysis would give rise to 6,10-dideoxyaucubin tetraacetate (6a). Stereospecific hydrogenation of the latter could yield 8(S)-6,10-dideoxy-7,8-dihydroaucubin tetraacetate (7a). With regard to the first step, Birch reduction of 3a has been reported to give 6a in moderate yield.[8] Formic acid-palladium on carbon has in our hands proved to be a useful system for catalytic transfer hydrogenation of allylic acetoxy[9] groups and we tested this in the present case. Thus treatment of 3a with 2 moles of formic acid in dioxane with palladium on carbon as the catalyst gave rise to a 33 % yield of 6a, after chromatography. However, using three moles of formic acid, a 46 % yield of 7a was obtained by simple crystallisation of the mixture of reaction products. Furthermore, none of the expected 8(R)-7a could be detected in the product. Reduction of double bonds under similar conditions have some preced-

ence.[10]

The reaction conditions used in Table 1, entry 9, above was applied to 6a, which proved to be a much better substrate, as 8a was obtained in a yield of 89 % after only 24h. This might be explained by the absence in 6a of the 6-acetoxyl group, which could cause steric hindrance. The reaction conditions developed in order to optimize the utilization of DMF (table 1, entry 8) were applied to 7a with some additional modifications. Thus the excess of DMF was



further reduced, and the reaction time was optimized to 48 h (by working up small samples every 4 h, and analysing by NMR). Here 9a was obtained in a yield of 26 %, but the utilization of DMF amounted to 27 %. This synthesis was repeated with ^{13}C-carbonyl DMF, giving [11-^{13}C]-9a in a similar yield. Though the yield of labelled 9a was as expected, it was observed by both ^{1}H and ^{13}C NMR spectra of the product that about 5 % of the labeling in the molecule was situated at C-3. We have not been able to provide a satisfactory explanation for this, since C-3 and C-11 at no stage during the reaction will become equivalent, according to the mechanism proposed.[11]
Iridoids carrying an 11-^{13}CHO group are very useful for the preparation of other labelled iridoids for use in biosynthetic experiments, as the aldehyde functionality can easily be either reduced or oxidized.

EXPERIMENTAL.

General: Melting points are corrected. NMR spectra were recorded on Bruker AM-500, HX-90 or WH-90 Instruments. TMS was used as internal standard in $CDCl_3$ and HDO (δ 4.8) in D_2O. HPLC was performed on Merck Fertigseulen size B and C (reversed phase), and flash chromatography on Si-gel (Merck, Kieselgel 60, 40-63 μ). Microanalyses were performed by Novo Microanalytical Laboratory. Acetylations were done with excess acetic anhydride in pyridine using 4-(dimethylamino)-pyridine as catalyst. Solvents and reagents used in the Vilsmeier reactions were freshly destilled. Dimethyl formamide (DMF) and CH_2Cl_2 were dried over 4 Å molecular sieve. The glass equipment was dried at 120 °C and the starting material at 60 °C for 24 hr. The 15 ml screw-cap vessel was sealed with a septum made of one layer of teflon foil and two butyl rubber layers, supported by a metal disc (with a central hole of 1.0 mm) and closed with a 13 mm plastic screw cap [12]. Aucubin (3) was obtained from *Aucuba japonica*.

General procedure for the Vilsmeier reaction (Table 1 entries 1-7).
DMF and $POCl_3$ were mixed in about one third of the solvent under N_2 at 0 °C in a 50 ml flask equipped with magnetic stirring and a reflux condenser sealed with a $CaCl_2$ drying tube. Stirring was continued for 1 h at r.t., then a solution of 3a (100 mg) in the remaining part of the solvent (1-3 ml) was

added, and the mixture heated on an oil bath to the desired temperature. Work up: The reaction mixture was poured into a vigorously stirred mixture of CH_2Cl_2 (20 ml) and a solution of Na_2HPO_4 in water (20 ml). 1 N NaOH was added dropwise to keep pH > 8. Stirring was continued for 1 h, then the phases were separated and the water phase extracted twice with CH_2Cl_2 (30 ml). The combined organic phases were washed with water (2x100 ml), dried with Na_2SO_4 and reduced in *vacuo*. Analysis of the products formed (entry 2): Preparative TLC (Et_2O) on the mixture of products gave in decreasing order of mobility: 4a (25 mg, 38 %); ^{1}H NMR ($CDCl_3$, 90 MHz): δ 6.31 (d, J=3.5 Hz, H-1); 5.61 (t, J=9.5 Hz, H-3); 5.16 (t, J=9.0 Hz, H-4); 5.08 (dd, J=3.5 and 9.5 Hz, H-2); 4.27 (m, H-5 and 6-CH2); 2.0-2.15 (4xOAc). 3a (20 mg, 20 %) and 5a (16 mg, 15 %), NMR-spectrum as below.

Preparation of 5a (*Table 1 entry* 8).
The procedure was the same as below, but with the parameters listed in Table 1 entry 8.

Preparation of 5a (*Table 1 entry* 9).
DMF (4.0 ml, 52.0 mmol), CH_2Cl_2 (4 ml) and 4 Å molecular sieve (0.7 g) were placed under dry Ar in the 15 ml screw-cap vessel. The solution was stirred for 1h, and then cooled to -25 °C. Then $POCl_3$ (2.4 ml, 26.0 mmol) was injected and the solution was stirred, without cooling, allowing the temperature to rise to r.t. when the stirring was continued for 1 h. Then a solution of aucubin hexaacetate (3a) (1.61 g, 2.67 mmol) in CH_2Cl_2 (4.0 ml) was injected. The pressure in the vessel was reduced to atm. pressure under Ar, and it was heated to 42 °C in an aluminum block, still with magnetic stirring, for 72 h. The reaction mixture was poured into a vigorously stirred mixture of CH_2Cl_2 (50 ml), satd. $NaHCO_3$ solution (50 ml) and solid $NaHCO_3$ (10 g). After 1 h the mixture was filtered through Celite. The product was extracted with CH_2Cl_2 (75 ml) washed with water (2x100 ml), dried over Na_2SO_4 and reduced in *vacuo*. Flash chromatography (hexane/EtOAc, 1:1) gave (5a) (1.05 g, 63 %). An analytical sample was crystallised from EtOH, m.p. 165-9°C (dec.); $[\alpha]_D^{20}$-85.0° (c=0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.32 (s, H-11); 7.17 (d, J=0,7 Hz, H-3); 5.91 (t, J=1.6 Hz, H-7); 5.52 (m, H-6); 5.27 (d, J=5.2 Hz, H-1); 4.88 (d, J=8.0 Hz, H-1'); 4.73 (m, 10-CH_2); 3.26 (dd, J=2.5 and 8.5 Hz, H-9); 3.18 (m, H-5); 2.1-1.95 (6xOAc); ^{13}C NMR ($CDCl_3$, 22.6 MHz): δ 189.5 (d, J=175 Hz, C-11); 170.5, 170.2, 170.1, 169.3, 168.9 (C=O, acetate); 160.5 (d, J=191 Hz, C-3); 142.5 (s, C-8); 129.5 (d,J=172 Hz, C-7); 121.1 (d, J=24 Hz, C-4); 96.5 (d, obsc, C-1); 80.4 (d, J=156 Hz,C-6); 61.1 (t, J=149 Hz, C-10); 45.6 (d, J=139 Hz, C-9); 38.0 (d, J=140 Hz, C-5); 21.1 and 20.5 (q, J=131 Hz, acetate-CH_3); 96.5, 72.2, 72.2, 70.6, 68.1 and 61.5 (C-1', C-3', C-5', C-2', C-4' and C-6'). Analysis: Found C, 53.71; H, 5.45; $C_{28}H_{34}O_{18}$ requires C, 53.67; H, 5.47.

Preparation of 6,10-dideoxy aucubin tetraacetate (6a).
Aucubin hexaacetate (3a) (3.6 g, 6.0 mmol) was dissolved in dry 1,4-dioxane (50 ml). Then Pd/C (750 mg) and HCOOH (510 mg, 11.1 mmol) was added. The stirred mixture was refluxed, and the progress of the reaction was followed by TLC. After 1 h the reaction mixture was cooled to r.t., filtered through celite and reduced in *vacuo*. Flash chromatography (EtOAc/Toluene 2:3) followed by crystallisation from EtOH provided 6a (850 mg, 30 %), m.p.134-7 °C, $[\alpha]_D^{20}$ -108° (c=0.4, $CHCl_3$), Lit.[8]: m.p. 137-8 °C, $[\alpha]_D^{20}$ -142° (c=0.5, $CHCl_3$).

Preparation of 8(S)-6,10-dideoxy-7,8-dihydroaucubin tetraacetate (7a).
Aucubin hexaacetate (3a) (10.0 g, 16.8 mmol) was dissolved in dry 1,4-dioxane (150 ml), Pd/C (2.5 g) and formic acid (1.9 ml, 50.4 mmol) was added, and the stirred mixture was refluxed for 1 h. Work up as above followed by crystallisation and recrystallisation of the crude mixture from EtOH gave 7a (3.70 g, 46 %) m.p. 126-7 °C; $[\alpha]_D^{20}$ -137.5° (c=0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.04 (dd, J=6.2 and 2.0 Hz, H-3); 5.16 (d, J=2.5 Hz, H-1); 4.90 (d, J=8.0 Hz, H-1'); 4.62 (m, H-4); 2.60 (m, H-5); 2.07-2.0 (4xOAc); 1.95-1.15 (7-CH_2; 6-CH_2; H-8; H-9); 1.04 (d, J=6.5 Hz, 10-CH_3) ; ^{13}C NMR ($CDCl_3$, 22.6 MHz) δ 170.2, 169.8, 169.0 and 168.9 (C=O, acetate); 137.1 (d, J=196 Hz, C-3); 108.4 (d, J=167 Hz, C-4); 93.5 (d, J=178 Hz C-1); 48.5 (d, J=131 Hz, C-9); 33.4 (d, J=127 Hz, C-8); 32.1 (t, J=129 Hz, C-7); 31.5 (d, J=135 Hz, C-5); 30.0 (t, J=131 Hz, C-6); 20.3-20.2 (q, J=130 Hz, acetat-CH_3); 19.3 (q, J=126 Hz, C-10); 95.2, 72.2, 71.6, 70.4, 68.1, 61.5 (C-1', C-5', C-3', C-2', C-4', and C-6'). Analysis: Found C, 56.90; H, 6.63; $C_{23}H_{32}O_{11}$ requires C, 57.02; H, 6.66.

Preparation of 8a.
Formylation of 6,10-dideoxy aucubin acetate (6a) (1.30 g, 2.70 mmol) with DMF (4 ml, 52 mmol) and $POCl_3$ (2.4 ml, 26 mmol) was performed as above in the preparation of 5a (table 1 entry 9) but with a reaction time of only 24 h. Flash chromatography (EtOAc/toluene, 2:3) gave 8a (1.23 g, 89%). From EtOH

was crystallised 1150 mg, m.p. 135-6 °C, $[\alpha]_D^{20}$ -28° (c=0.7, $CHCl_3$). Analysis: Found C, 56.07; H, 6.03; $C_{24}H_{30}O_{12}$ requires C, 56.46; H, 5.92.

Preparation of iridotrialglucoside (9) with high utilization of DMF. 7a (3.48 g, 7.19 mmol) in CH_2Cl_2 (6 ml) was formylated as above (preparation of 8a (Table 1 entry 9)) using DMF (0.50 g, 6.88 mmol) and $POCl_3$ (1.0 g, 6.50 mmol) under dry Ar for 48 h. DMF and $POCl_3$ were mixed in 2 ml of the solvent. At aprox. -8 °C the Vilsmeier complex precipitated, but dissolved again at about +5 °C. Flash chromatography of the crude product (hexane/EtOAc, 1:1) gave 9a (1.85 g contaminated with glucose tetraacetate). De-acetylation (NaOMe/MeOH) and purification by prep-HPLC (H_2O/MeOH,3:2), yielded 9 (682 mg, 27 %). From EtOAc was crystallised a sample, m.p. 146-8 °C; $[\alpha]_D^{20}$ -104° (c=0.6, MeOH). Lit.[13]: m.p. 146-7 °C, $[\alpha]_D^{20}$ -117° (c=2.0, MeOH); ^{1}H NMR (D_2O, 500 MHz): δ 9.13 (s,H-11); 7.45 (d,J=3.9 Hz,H-3); 5.54 (d,J=3.9 Hz,H-1); 4.86 (d,J=8.0 Hz,H-1'); 2.95 (bq, J=6 Hz, H-5); 2.2-1.25 (H-9, H-8, 6-CH_2 and 7-CH_2); 1.09 (d, J=6.1 Hz, 10-CH3); ^{13}C NMR (D_2O, 22.6 MHz): δ 196.0 (C-11); 164.8 (C-3); 125.3 (C-4); 98.9 (C-1); 48.4 (C-9); 35.4 (C-8); 33.2 (C-7); 31.3 (C-5); 30.8 (C-6); 19.9 (C-10); 99.7, 77.2, 76.5, 73.5, 70.4 and 61.5 (C-1', C-5', C-3', C-2', C-4' and C-6'). Analysis: Found C, 53.02; H, 7.16; $C_{16}H_{24}O_8.H_2O$ requires C, 53.02; H, 7.25.

Preparation of [11-^{13}C]-iridotrialglucoside (9). Formylation of (7a) (3.48 g, 7.19 mmol) as above, using 0.5 g > 90 % ^{13}C-carbonyl DMF (Stohler Isotope Chemicals) gave 1.82 g crude [11-^{13}C]-9a, after flash chromatography. Deacetylation (NaOMe/MeOH) and purification by prep-HPLC as above, gave [11-^{13}C]-12 (672 mg, corresponding to a total utilization of 27 % of the ^{13}C-DMF) as a colourless foam. ^{1}H NMR (D_2O, 500 MHz): δ 9.13 (d, $J_{H,C}$=175 Hz, H-11 on ^{13}C-11); δ 9.13 (s, H-11 on ^{12}C-11) (integration showed an 86 % labelling of C-11); 7.45 (bd, $^3J_{H\text{-}3,C\text{-}11}$ = 5.3 Hz, H-3 β to ^{13}C-11); 7.45 (bs, H-3 β to ^{12}C-11); 7.45 (bd, $^1J_{H\text{-}3,C\text{-}3}$ = 191 Hz, H-3 on ^{13}C-3) (integrals showed 5 % labelling of C-3); the remaining part of the spectrum as above; ^{13}C NMR (D_2O, 22.6 MHz): δ 196.2 (C-11); 164.8 (C-3) (relative intensities: 14:1).

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