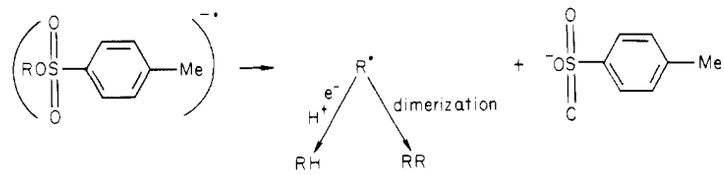
Scheme I



under heterogeneous conditions (Table I and ref 3). However, other differences in the product distribution cannot be ascribed, on the basis of the available data, to the different methods, the heterogeneous or the homogenous one, used. In fact, for example, *p*-methylbenzyl tosylate gave 1,2-bis(*p*-methylbenzyl)ethane only in the reduction by sodium naphthalene, while both the cathodic and the sodium anthracene reduction[3] gave only *p*-methylbenzyl alcohol and *p*-xylene (Table I).

In conclusion, it is clear that after reduction of the tosylate to its radical anion, cleavage will occur along the weakest bond, giving a delocalized radical. This is the case of benzyl, benzhydryl, and allyl tosylate, where a stabilized alkyl radical R· is formed. The latter can then be further reduced to the hydrocarbon RH (Scheme I). There is, in fact, ample precedent to such a behavior with both benzyl benzoates[6] and benzyl ethers and acetates.[7]

The factors leading to the ethane derivatives RR (Table I) can be less clearly identified on the basis of the present data. In fact, among the factors leading to production of ethane derivatives, it is clear that not only does the nature of the reducing agent (heterogeneous vs. homogeneous) play an important role, but so does the strength of the reducing agent under homogeneous conditions (see Table I). Whether in the homogeneous reduction of tosylates the ethane derivatives R–R come from dimerization[3] of the radical R· or rather from attack of the carbanion R^- on unreacted tosylate has yet to be established.

Experimental Section

In order to aid comparison of data for homogeneous and heterogeneous reductions, we include here experimental details for our previous electrochemical reduction of the present tosylates.[3]

Chemicals. The tosylates were synthesized according to reported procedures: cyclohexyl-,[8] *p*-methylbenzyl,[9] benzhydryl-,[10] and allyl tosylate.[11] Reaction products were isolated and compared with authentical samples: cyclohexanol (Merck), *p*-methylbenzyl alcohol (Merck), benzhydrol (Fluka), allyl alcohol (Merck), *p*-xylene (C. Erba), diphenylmethane (Merck), propene (Merck), 1,2-di-*p*-benzylethane,[12] 1,2-dibenzhydrylethane,[13] bis(*p*-methylbenzyl) ether,[14] and dibenzhydryl ether.[8] Tetrahydrofuran (THF) (C. Erba) was distilled from lithium aluminum hydride. Dimethylformamide (DMF) (C. Erba) was distilled over molecular sieves. Acetonitrile (AN) (C. Erba) was distilled over P_2O_5 and then fractionated over calcium hydride. Tetraethylammonium perchlorate (TEAP) (C. Erba for polarography) was dried at 80° C in vacuo. Guanidinium perchlorate (GP) was prepared by treatment of guanidinium chloride with excess sodium hydroxide, extracting with ether, and neutralizing with $HClO_4$. The salt was dried in vacuo at 80 °C.

General Procedure for the Reduction of Tosylates by Radical Anions. The method of ref 4 was closely imitated using a 4:1 molar excess of the radical anion over the tosylate.

General Procedure for the Cathodic Reduction of Tosylates. Electrolyses were carried out at constant potential by means of an AMEL model 552 potentiostat coupled to an AMEL 721 digital integrator. Anodic and cathodic compartments were separated by means of a glass frit. The working electrode was a mercury pool. The auxiliary electrode was a platinum wire which was flat coiled parallel to the mercury pool. The reference electrode was silver–0.1 M silver perchlorate in AN. Electrolysis potentials are referred to the saturated calomel electrode (SCE). The electrolysis solution, which was accurately flushed with dry nitrogen, was stirred by a magnetic bar. The extent of the electrolysis was monitored by cyclic voltammetry on a dropping mercury electrode which was lodged within the electrolytic cell. The supporting electyrolyte was 0.1 M. Data for the electrolyses[3] are reported in Table II.

Acknowledgment. Financial support by C.N.R., Roma, is gratefully acknowledged.

Registry No. Cyclohexyl tosylate, 953-91-3; *p*-methylbenzyl tosylate, 4606-98-8; benzhydryl tosylate, 5435-24-5; allyl tosylate, 4873-09-0; cyclohexanol, 108-93-0; toluene, 108-88-3; *p*-methylbenzyl alcohol, 589-18-4; *p*-xylene, 106-42-3; bis(*p*-methylbenzyl)ethane, 7568-23-2; bis(*p*-methylbenzyl) ether, 38460-98-9; benzhydrol, 91-01-0; diphenylmethane, 101-81-5; 1,1′,2,2′-tetraphenylethane, 632-50-8; dibenzhydryl ether, 574-42-5; allyl alcohol, 107-18-6; propene, 115-07-1; sodium naphthalene, 3481-12-7; sodium anthracene, 12261-48-2; 1,5-hexadiene, 592-42-7; benzhydryl formate, 66680-81-7.

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Formation of CH_2Cl_2-Soluble Urea Derivatives during Solid-Phase Peptide Synthesis with Unsymmetrical Carbodiimides

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Dicyclohexylcarbodiimide (DCC) (Ia) is by far the most utilized coupling reagent in solid phase peptide synthesis.[1] Yet, with DCC it is difficult to wash out the precipitated dicyclohexylurea IIa before the next deprotection step[2] due to its poor solubility in CH_2Cl_2. Unfortunately, IIa, which remains inside the resin, is most soluble in solvents like alcohols that shrink the resin. In any case, these washings can only be performed after the coupling. Last but not least, these washings are time and solvent consuming, and the polymer has to be thoroughly washed afterwards with CH_2Cl_2 to prevent side reactions.[3]

In 1976, Sarantakis[4] briefly reported the synthesis of a cyclic undecapeptide, using diisopropylcarbodiimide (Ib).

(1) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
(2) Dicyclohexylurea is soluble in a mixture of trifluoroacetic acid and CH_2Cl_2.
(3) C. Birr, "Aspects of the Merrifield Peptide Synthesis", Springer-Verlag, Berlin, 1978.
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Table I. Coupling Efficiencies of Ia to Ie and Solubility of IIa to IIe

	coupling efficiency,[a] %		urea solubility in CH_2Cl_2, g/L
	Ala-resin + Boc-Phe	Phe-resin + Boc-Val	
Ia	95	77	1.5
Ib	96	76	3.5
Ic	2[illegible]	10	400
Id	97	74	140
Ie	96	72	330

[a] Based on the quantity of Boc-amino acid and diimide (0.75 equiv with respect to the loading of the resin).

Scheme I

$$R_1N{=}C{=}NR_2 \xrightarrow{\text{coupling}} R_1NHCNHR_2$$
I II

I,II a, $R_1 = R_2$ = cyclohexyl
b, $R_1 = R_2$ = isopropyl
c, R_1 = phenyl; R_2 = ethyl
d, R_1 = benzyl; R_2 = isopropyl
e, R_1 = benzyl; R_2 = ethyl

Table II. Properties of Ic, Id, and Ie

	bp, °C	yield, %	IR, cm^{-1} -N=C=N-	RMN R_1	RMN R_2	
Ic	64 (0.02 mm)	60	2130	6.8-7.4 (5 H, m)	3.2 (2 H, q), 1.15 (3 H, t)	J = 7.5 Hz
Id	78-85 (0.1 mm)	65	2120	7.1-7.4 (5 H, m), 4.3 (2 H, s)	3.4 (1 H, sept), 1.0 (6 H, d)	J = 6.8 Hz
Ie	63 (0.1 mm)	64	2120	7.1-7.4 (5 H, m), 4.3 (2 H, s)	3.05 (2 H, q), 1.05 (3 H, t)	J = 7.5 Hz

The urea derivative IIb was described as more soluble in organic solvents and more easily rinsed out. In our hands, IIb proved to be only slightly more soluble in CH_2Cl_2 than Ib, so we decided to undertake a systematic study of this problem.

We synthesized several urea derivatives and determined their solubility in CH_2Cl_2. We observed that while some ureas exhibit poor solubilities in CH_2Cl_2 others are quite soluble; among them were three unsymmetrical ureas IIc, IId, and IIe.

We also checked the coupling efficiency of the corresponding diimides: Ia, Ib, Ic, Id, and Ie. Diimides Ic, Id, and Ie were selected because Ito[5] reported that when a peptide was synthesized in solution by the use of an unsymmetrical diimide, the formation of *N*-acylurea was markedly suppressed, and racemization was reduced in comparison to the use of DCC.

Couplings were performed by treating the aminoacyl resin in CH_2Cl_2 with only 0.75 equiv of Boc amino acid and diimide with respect to the loading of the resin. After 1 h of coupling, the amount of remaining unreacted amino groups on the polymer was determined by the picrate method.[6] These experiments were performed with two different coupling reactions: alanyl resin + Boc-phenylalanine and phenylalanylresin + Boc-valine. We report the results in Table I.

Conclusions

Ib has the same coupling efficiency as DCC but gives IIb which is only slightly more soluble than IIa.

Ic gives the very soluble urea IIc, but its coupling efficiency is drastically decreased due to conjugation of the diimide group with the aromatic ring.

Id and Ie give ureas which are very soluble in CH_2Cl_2 and exhibit coupling efficiencies which are only slightly reduced in one case. It must be emphasized that solubility of IId and IIe is sufficient to prevent precipitation during the coupling reaction.

These results indicate that in order to avoid the formation of an insoluble urea derivative the unsymmetrical diimides Id and Ie can be used instead of DCC for coupling in the solid phase peptide synthesis.

Experimental Section

Materials and Methods. Boc-amino acids were purchased from Serva; diisopropylamine (DIEA), trifluoracetic acid (TFA), dicyclohexylcarbodiimide, and diisopropylcarbodiimide were from Aldrich. Chloromethylated copolystyrene 1% divinylbenzene resin (Bio Beads SX1, 200–400 mesh, 1.25 mequiv of chlorine per g) was obtained from Bio-Rad. Methylene chloride, technical grade, was distilled from K_2CO_3.

***N*-Benzyl-*N'*-isopropylurea (IId).** To a stirred solution of 12.6 g (0.12 mol) of benzylamine in 50 mL of Et_2O was added dropwise a solution of 10 g (0.12 mol) of isopropyl isocyanate in 50 mL of Et_2O. After 30 min, the precipitated urea was collected by filtration, washed with Et_2O, and dried to give 21 g of IId, mp 103–104 °C (yield = 91%).

Other substituted ureas were prepared from the appropriate isocyanates and amines in a similar manner.

Benzylisopropylcarbodiimide (Id). A solution of 10 g (0.05 mol) of IId and 10.6 g (0.1 mol) of triethylamine in 80 mL of CH_2Cl_2 was added dropwise to a solution of 19 g (0.1 mol) of tosyl chloride in 50 mL of CH_2Cl_2, and the resulting mixture was refluxed for 4 h. After being cooled and filtered, the solution was treated with 20 g of Na_2CO_3 in 50 mL of H_2O and stirred for 30 min. The aqueous phase was extracted twice with CH_2Cl_2, and the combined extracts were washed with H_2O, dried (Na_2SO_4), and evaporated. The residue was dissolved in 50 mL of Et_2O, filtered, and evaporated. Distillation under vacuum gave a colorless liquid: bp 78–85 °C (0, 1 mm); yield = 65%.

Other substituted carbodiimides (Table II), Ic and Ie, were prepared from appropriate ureas IIc and IIe in a similar manner.

Solid-Phase Synthetic Procedure. Esterification of the first Boc-amino acid to the resin was performed as described by Gisin.[7] The amount of amino acid on the resin was 0.89 mequiv/g of Boc-alanyl resin and 0.85 mequiv/g of Boc-phenylalanyl resin.

The synthetic procedure including the picric titration was carried out on 1 g of Boc-aminoacyl resin in the automated Beckman synthesizer Model 990 B, according to the following protocol: (1) 3 × 3 nm CH_2Cl_2, 20 mL; (2) 1 × 3 nm 50% TFA–CH_2Cl_2, 18 mL; (3) 1 × 30 nm 50% TFA–CH_2Cl_2, 18 mL; (4) 3 × 3 nm CH_2Cl_2, 18 mL; (5) 2 × 3 nm EtOH, 20 mL; (6) 4 × 3 nm CH_2Cl_2, 20 mL; (7) 3 × 3 nm 5% DIEA–CH_2Cl_2, 20 mL; (8) 4 × 3 nm CH_2Cl_2, 20 mL; (9) 1 × 3 nm 0.75 equiv of Boc-amino acid–CH_2Cl_2, 10 mL; (10) 1 × 60 nm 0.75 equiv of diimide–CH_2Cl_2, 10 mL; (11) 2 × 3 nm CH_2Cl_2, 20 mL; (12) 2 × 3 nm EtOH, 20 mL; (13) 4 × 3 nm CH_2Cl_2, 20 mL; (14) 3 × 3 nm 5% DIEA–CH_2Cl_2, 20 mL; (15) 2 × 3 nm CH_2Cl_2, 20 mL; (16) 2 × 3 nm EtOH, 20 mL; (17) 4 × 3 nm CH_2Cl_2, 20 mL; (18) 3 × 3 nm 0.1 M picric

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(6) B. F. Gisin, *Anal. Chim. Acta*, **58**, 248 (1972).
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acid–CH_2Cl_2, 20 mL; (19) 2 × 3 nm CH_2Cl_2, 20 mL; (20) 2 × 3 nm EtOH, 20 mL; (21) 4 × 3 nm CH_2Cl_2, 20 mL; (22) 3 × 3 nm 5% DIEA–CH_2Cl_2, 20 mL; (23) 4 × 3 nm CH_2Cl_2, 20 mL. Washes in steps 22 and 23 were collected and their absorbance was measured at 362 nm.

Registry No. Ia, 538-75-0; Ib, 693-13-0; Ic, 21002-18-6; Id, 38463-75-1; Ie, 63796-18-9; IIa, 2387-23-7; IIb, 4128-37-4; IIc, 6957-05-7; IId, 71819-34-6; IIe, 61843-91-2; ethyl isocyanate, 109-90-0; ispropyl isocyanate, 1795-48-8; benzenamine, 62-53-3; benzylamine, 100-46-9.

Communications

Synthesis of Brassino Steroids: New Plant-Growth-Promoting Steroids

Summary: We have synthesized two highly physiologically active brassino steroids, structural isomers of brassinolide (2α,3α,22(*R*),23(*R*)-tetrahydroxy-24(*S*)-methyl-*B*-homo-7-oxa-5α-cholestan-6-one), the recently characterized plant growth promoting steroid isolated from rape pollen.

Sir: Lipoidal substances that have growth-promoting effects on plants have been extracted from the pollen[1] of rape (*Brassica napus* L.). One of these plant growth promoters, brassinolide, has been identified by physical methods, including X-ray analysis,[2] which showed it to be 2α,3α,22(*R*),23(*R*)-tetrahydroxy-24(*S*)-methyl-*B*-homo-7-oxa-5α-cholestan-6-one (**1**). We now wish to report the first synthesis of two 22,23 isomeric brassino steroids and a nonlactonic steroid with plant-growth-promoting activity.

Solvolysis of ergosterol tosylate (**2**) according to reported methods[3] gave i-ergosterol (**3**) (Scheme I). Oxidation of **3** to **4** with chromic acid in pyridine[4,5] followed by the reduction of **4** with lithium and liquid ammonia yielded compound **5**. Acid rearrangement of **5** by refluxing it for 2 h in acetic acid–5 N sulfuric acid[5] (20 mL/5 mL per gram of the ketone[6]) followed by saponification of the resulting acetate gave 3β-hydroxy-24β-methyl-5α-cholest-22-en-6-one (**6a**). Compound **6a**, mp 186–187 °C, $[\alpha]^{25}_D$ −35°, was obtained from ergosterol without purification of any intermediates in an overall 33% purified yield via the described sequence of reactions.

The detosylation of **6b** in dimethylformamide, which contained 10% each of lithium bromide and **6b** at reflux temperature for 45 min, gave **7** in 70% purified yield, mp 123–124 °C, $[\alpha]^{25}_D$ +3°. Treatment of **7** for 3 days at room temperature in dry benzene (60 mL/g) that contained a trace of pyridine and 2 molar equiv of osmium tetroxide gave nearly quantitative yield on reductive cleavage of the osmate ester 1:1 mixture[7] of the tetrahydroxy ketones **8a** (R_f 0.53) and **9a** (R_f 0.45)[8] with the expected 2α,3α-*cis*-diol orientation.[9] The tetrahydroxy ketones were separated by column chromatography over Woelm neutral alumina (activity grade III) in a gradient-type elution system progressing from chloroform–benzene (90:10) through chloroform–methanol (1:1). The tetrahydroxy ketone (**8a**), with an R_f value of 0.53, was recrystallized from ethyl acetate, mp 182–183 °C, $[\alpha]^{25}_D$ −2°. A similar recrystallization of the component with an R_f value of 0.45 gave **9a**, mp 241–242 °C, $[\alpha]^{25}_D$ 0°.

A Baeyer–Villiger oxidation[10] of the tetraacetates **8b** and **9b** in chloroform with *m*-chloroperbenzoic acid for 2 weeks[11] at room temperature gave predominantly the crude tetraacetoxy-7-oxa ketones **10a** and **11a**, respectively. Both lactones also contained a small quantity of the respective isomeric 6-oxa ketone. The lactones **10a** and **11a** were purified by column chromatography over Unisil[12] by initially eluting the columns with benzene–chloroform (90:10) and then with increasing percentages of chloroform in benzene. Saponification of **10a** with 4% potassium carbonate in refluxing 70% aqueous methanol for 4 h, followed by acidification with dilute hydrochloric acid solution, and subsequent recrystallization of the precipitate from ethyl acetate gave (in 25% overall purified yield from **7**) 2α,3α,22β,23β-tetrahydroxy-24β-methyl-*B*-homo-7-oxa-5α-cholestan-6-one (**10b**): mp 194–195 °C; $[\alpha]^{25}_D$ +31°; NMR (C_5D_5N) δ 4.1 (2, d, 7a-H, J = 4 Hz), 0.63 (3, s, 18-H), 1.03 (3, s, 19-H); EI–MS showed no M^+ at m/e 480, the first observable peak at m/e 462 ($M^+ - H_2O$, <1), and other ions at m/e 447 (3), 409 (3), 380 ($M^+ - 100$, 27) results from cleavage of 22,23 carbon bond, 362 (20), 350 (24), 343 (15), 333 (14), 319 (10), 303 (12), 285 (11), 208 (14), 189 (19), 177 (25), 107 (52), 81 (77), 71 (58), 43 (100); CI–MS (isobutane) showed ions at m/e 481 (M + 1), 463 (M + 1 − H_2O), 445 (M + 1 2 H_2O), 427 (M + 1 − 3H_2O), 409 (M + 1 − 4H_2O), 379, 361, 349, 321, 303.

(1) (a) J. W. Mitchell, N. Mandava, J. F. Worley, J. R. Plimmer, and M. V. Smith, *Nature (London)*, **225**, 1065 (1970); (b) J. F. Worley and J. W. Mitchell, *J. Am. Hortic. Soc.*, **96**, 270 (1971); (c) J. F. Worley and D. T. Krizek *Hortic. Sci.*, **7**, 480 (1972); (d) J. W. Mitchell and L. E. Gregory, *Nature (London), New Biol.*, 239 (1972); (e) N. Mandava, M. Kozempel, J. F. Worley, D. Matthees, J. D. Warthen, Jr., M. Jacobson, G. L. Steffens, H. Kenny and M. D. Grove, *Ind. Eng. Chem. Prod. Res. Dev.*, **17**, 351 (1978).

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(3) W. R. Nes and J. A. Steele, *J. Org. Chem.*, **22**, 1457 (1957).

(4) M. J. Thompson, C. F. Cohen, and S. M. Lancaster, *Steroids*, **5**, 745 (1965); in this reference concerning the solvolysis of ergosterol tosylate, potassium bicarbonate was used, not potassium carbonate as reported. All compounds here and thereafter isolated and recrystallized were characterized by spectral methods (IR, NMR, MS) and showed only a single component by TLC and other chromatographic methods.

(5) G. H. R. Summers, *J. Chem. Soc.*, 4489 (1958).

(6) Since the intermediates **2** through **5** were not purified, the quantity of **5** present was based on the calculated weight of **4** by UV analysis.

(7) Hydroxylation of **6b** via the osmate ester also gave a 1:1 mixture of compounds from which compounds **8a** and **9a** were also obtained through further reactions of hydroxylated products of **6b**. Thus the mixture results from hydroxylation of the side chain.

(8) From silica gel TLC plate developed twice in the solvent system of chloroform–ethanol (7:1).

(9) L. Fieser and M. Fieser, "Steroids", Reinhold, New York, 1959, p 274.

(10) (a) R. C. Cookson, R. P. Gandhi, and R. M. Southam, *J. Chem. Soc. C*, 2494 (1968); (b) M. S. Ahmad, G. Moinuddin, and I. A. Khan, *J. Org. Chem.*, **43**, 163 (1978).

(11) The progress of the reaction was monitored by TLC.

(12) (a) With Unisil (silicic acid) as an adsorbent there is no loss of lactones during chromatography though removal of contaminants was less effective than with Woelm neutral alumina (activity Grade II). (b) Mention of a company name or proprietary product in this paper does not constitute an endorsement of the product by the U.S. Department of Agriculture.