66. Sambucinic Acid, a New Metabolite of *Fusarium sambucinum*

45th Communication on Verrucarins and Roridins[1])

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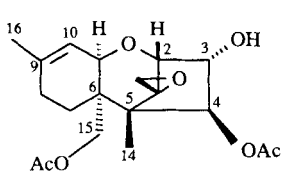
Sambucinic acid (**5**), a C_{15}-trichothecene with a modified structure, has been isolated from cultures of *Fusarium sambucinum*. The structure is established by spectroscopic data and X-ray diffraction analysis. A hypothetical biogenetic pathway is proposed.

The trichothecenes are a still growing class of closely related sesquiterpenoid secondary metabolites produced by moulds, especially various species of *Fungi imperfecti* [2]. Many members of the family display a wide range of biological effects, such as cytostatic activity, but they are also highly toxic.

Recently, we reported on the structure elucidation of sambucinol (**2**) and sambucoin (**3**) which were isolated from cultures of *Fusarium sambucinum* (ATCC No. 11852) as minor metabolites [3]. The major compound was diacetoxyscirpenol (anguidine, **1**). In the



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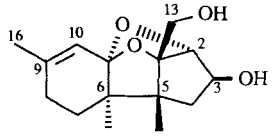

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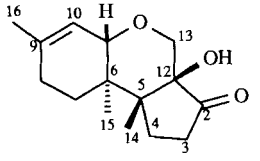

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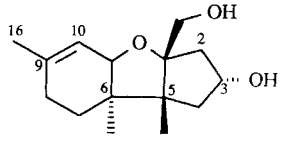

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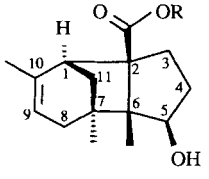

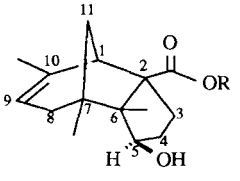

5 R=H
6 R=CH_3

[1]) 44th Communication: [1].

Table 1. *^{13}C- and ^{1}H-NMR Data for Sambucinic Acid* (**5**)[a])

Position	^{13}C Shift	^{1}H Shift	
1	46.76 (*d*)	2.69	(*d*, *J* = 4)
2	70.90 (*s*)	–	
3	28.19 (*t*)	1.72	(*td*, *J* = 12, 6)
		2.35	(*ddd*, *J* = 12, 6.5, 2)
4	38.47 (*t*)	2.27	(*dtd*, *J* = 11, 5.5, 1.5)
		2.67	(*m*)
5	75.70 (*d*)	4.44	(*dd*, *J* = 10.5, 5.5)
6	60.25 (*s*)	–	
7	42.54 (*s*)	–	
8	41.34 (*t*)	2.10	(*dquint.*, *J* = 18, 2.5)
		2.50	(*dm*, *J* = 18)
9	121.40 (*d*)	5.33	(*m*)
10	140.76 (*s*)	–	
11	46.11 (*t*)	1.82	(*d*, *J* = 11)
		2.90	(*ddd*, *J* = 10.5, 4, 1.3)
COOH	179.17 (*s*)	–	
C–C(6)	16.10 (*q*)	1.55	(*s*)
C–C(7)	25.41 (*q*)	1.15	(*s*)
C–C(10)	25.04 (*q*)	1.63	(*dt*, *J* = 4, 2)

[a]) The spectra were recorded at 100.58 MHz (^{13}C) and 400 MHz (^{1}H) in (D_5)pyridine. Chemical shifts are given in ppm relative to TMS (= 0.0).

same large-scale fermentation, we have found a third minor metabolite, which we name sambucinic acid (**5**). Whereas diacetoxyscirpenol (**1**) possesses the normal trichothecane skeleton, the minor metabolites **2**, **3**, and **5** as well as 'apotrichothecene'[2]) (**4**), a fourth minor metabolite isolated by a Canadian research group [5], are characterized by modified trichothecane skeletons.

Sambucinic Acid (= *5-Hydroxy-6,7,10-trimethyltricyclo[5.3.1.0^{2,6}]undec-9-ene-2-carboxylic acid;* **5**). Yield *ca.* 0.4 mg/l. M.p. 200–207°. IR (KBr): 3430, 3400, 3200–2700, 1695. The composition of $C_{15}H_{22}O_3$ was secured by elemental analysis (found: C 70.36, H 9.29, calc.: C 72.00, H 8.80) and by EI-MS: 250 (M^{+}), 232, 205, 141. CI-MS (NH_3): 268 ([M + NH_4]$^{+}$), 250 (M^{+}), 233. MS-CI (CH_4): 251 ([M + 1]$^{+}$), 233, 189.

The structure elucidation was achieved by NMR spectroscopy, using modern pulse sequences[3]). The ^{1}H- and ^{13}C-NMR assignments were made by a ^{1}H, ^{13}C heterocorrelation experiment *(Table 1, Fig. 1)*.

There are signals of two olefinic C-atoms (C(9), C(10)), a secondary alcohol (C(5)), and a CO group at 179.17 ppm, which must be a carboxylic acid. Treatment of **5** with CH_2N_2 let to methyl sambucinate (**6**) (EI-MS: 264 (M^{+}), 205, 155 (100). CI-MS (NH_3): 282 ([M + NH_4]$^{+}$), 264 (M^{+}), 247 (100)). In the ^{1}H-NMR spectrum, a *singlet* at 3.67 ppm appeared, as expected.

A ^{1}H,^{1}H-correlated 2D-NMR experiment *(Fig. 2)* with **5** clearly showed the following three isolated spin systems:

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[2]) The compound should correctly be named apotrichothec-9-ene-3α,13-diol, because apotrichothecane is the generic name for the skeleton [4].

[3]) We thank Dr. *Rüegger, Spectrospin,* Fällanden and Prof. *H. Fritz, Ciba-Geigy AG,* Basel, for recording the spectra and the helpful discussion.

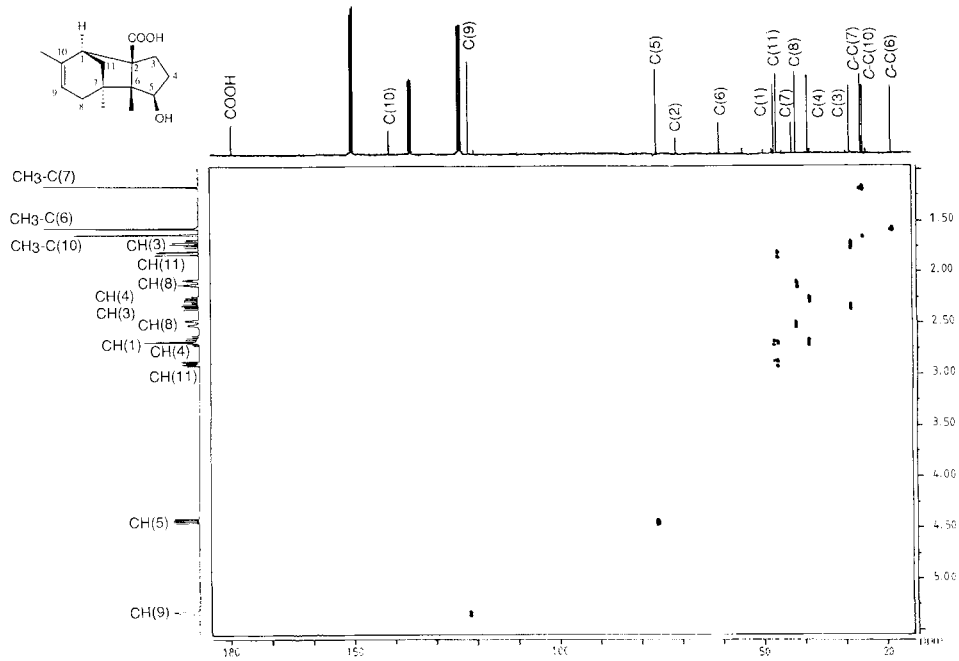


Fig. 1. *^{13}C, ^{1}H-Correlated 2D-NMR spectrum of sambucinic acid* (**5**) (for conditions, *cf. Table 1*)

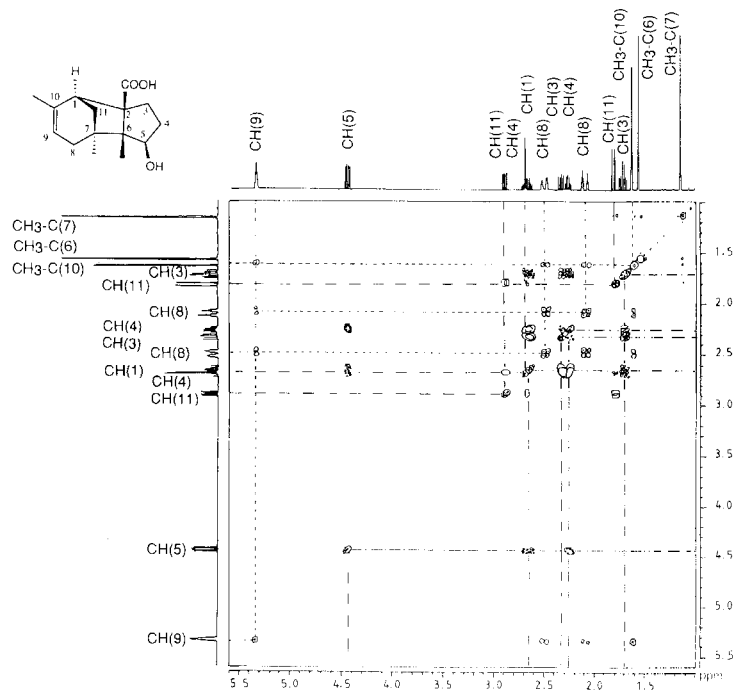


Fig. 2. *$^{1}H,^{1}H$-Correlated 2D-NMR spectrum of sambucinic acid* (**5**) (for conditions, *cf. Table 1*)

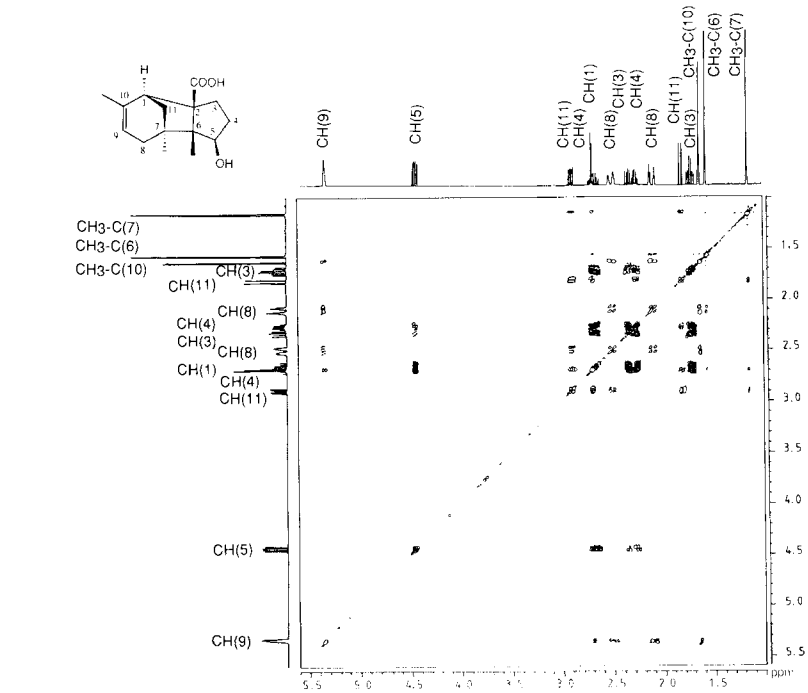


Fig. 3. *$^1H,^1H$ Long-range-correlated 2D-NMR spectrum of sambucinic acid* (**5**) (for conditions, *cf. Table 1*)

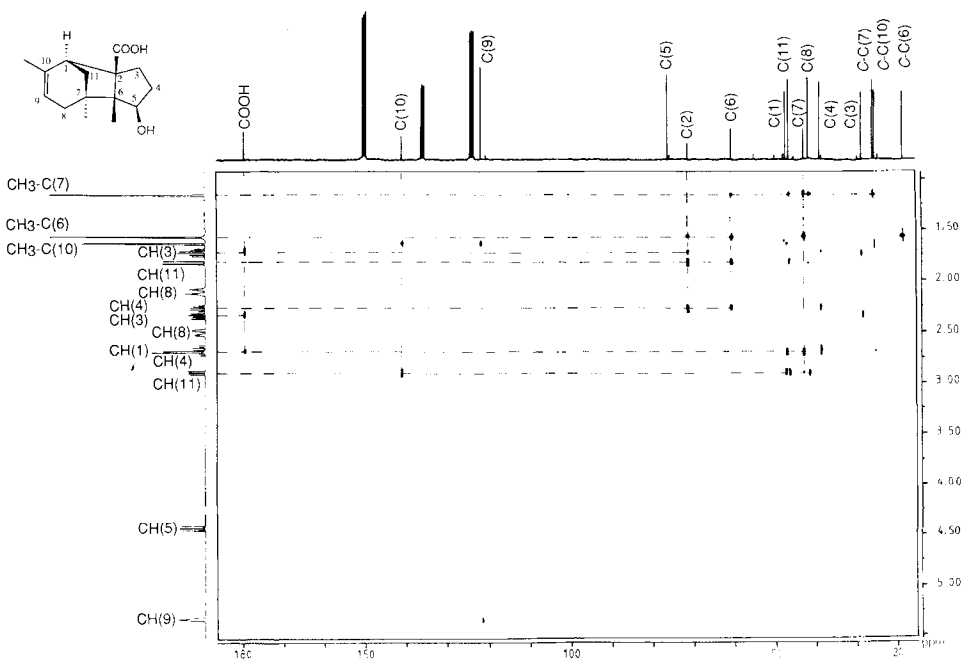
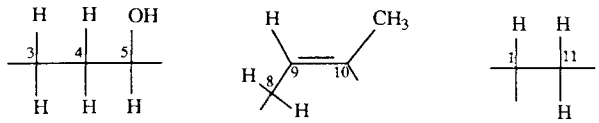


Fig. 4. *$^{13}C,^1H$ Long-range-correlated 2D-NMR spectrum of sambucinic acid* (**5**) (for conditions, *cf. Table 1*)



The connection of the three spin systems and the location of the quarternary C-atoms and Me groups were established by a $^1H,^1H$ long-range correlated 2D-NMR *(Fig. 3)* and a $^1H,^{13}C$ heterocorrelation long-range 2D-NMR *(Fig. 4)*: Long-range coupling of *C*OOH with C*H*(1) and CH_2(3) clearly shows the sequence CH_2(3)–C(2)(COOH)–CH(1). C(2) (70.9 ppm) couples with C*H*(11), C*H*(3), C*H*(4), and CH_3–C(6). C(6) (60.2 ppm) shows long-range coupling with CH_3–C(6) (1.55 ppm), CH_3–C(7) (1.15 ppm), C*H*(4), and C*H*(11). C(7) (42.5 ppm) also couples with CH_3–C(6) and CH_3–C(7) and also with C*H*(1) and C*H*(11).

These facts and various additional correlations allowed to propose the structural formula for sambucinic acid.

The relative configuration and the location of CH_3–C(10) was assigned by a NOESY *(Fig. 5)* experiment. Hence, the configuration of C(5) was derived from a NOE of C*H*(5) with CH_3–C(7) and the attachment of CH_3–C(10) at the double-bond was determined by a NOE of CH_3–C(10) with C*H*(1).

The proposed structure was proven by X-ray diffraction analysis. *Table 2* summarizes the crystal data, data collection details, and structure determination parameters. Unit-cell parameters were determined from accurate centering of 25 independent strong reflec-

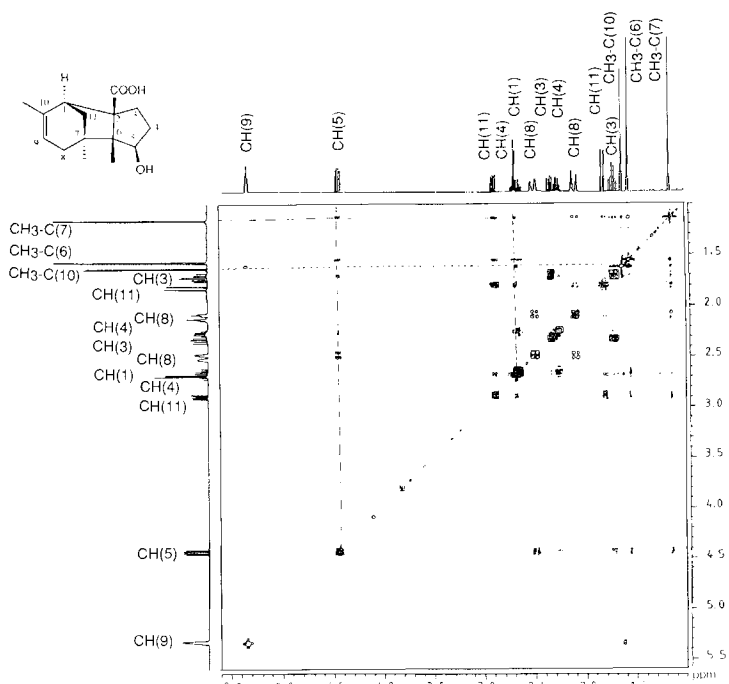


Fig. 5. *NOESY Spectrum of sambucinic acid* (**5**) (for conditions, *cf. Table 1*)

Table 2. *Crystal Data and Parameters of Data Collection for* **5**

Formula	$C_{15}H_{22}O_3$
Crystal system and space group	Orthorhombic, $P22_12_1$ (No. 18 in non-standard setting)
a [Å]	7.793(2)
b [Å]	13.992(3)
c [Å]	24.409(6)
α [deg]	90.0
β [deg]	90.0
γ [deg]	90.0
V [Å^3]	2650.2
Z	8 (2 indep. molec./asymm. unit)
Crystal size [mm]	0.2 × 0.1 × 0.1
Temp. [K]	293
Θ_{max} [deg]	27
Radiation	MoK_α (λ = 0.71069 Å)
Scan type	$\omega/2\,\Theta$
No. of independent reflections	3322
No. of reflections used in refinements	1268 ($F > 2\sigma(F)$)
No. of variables	333
Final R_w	0.0770
Weighting system	$1.62/(\sigma^2(F)+5.48\cdot10^{-4}F^2)$

tions by the least-squares method. Four standard reflections monitored every 3600 s during data collection showed no intensity loss. The row data set was corrected for polarization effects. No correction for absorbance was applied. The final data set yielded a total of only 1268 independent reflections according to the criterion $F_o > 2\sigma(F_o)$. Crystals of $C_{15}H_{22}O_3$ have generally a weak intensity at every *Bragg* angle. Since there was no significant scattering observable in *h00*, one could not distinguish between the space groups $P22_12_1$ and $P2_12_12_1$. Density determination by flotation and the metric of the unit cell (18.4 Å^3/non-H-atom) indicated two independent molecules per asymmetric unit. The

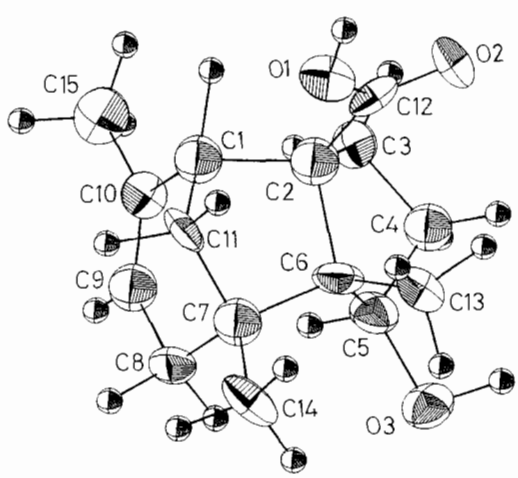


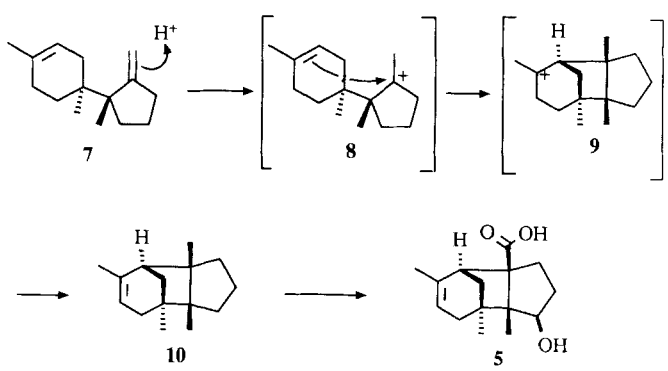
Fig. 6. *ORTEP Plot of sambucinic acid* (**5**) (arbitrary numbering)

programs MULTAN, MAGIC, YZARC led for both space groups to noninterpretable *E* maps, even when a large amount of starting phases were chosen. Finally, the structure was solved by the SHELXS-86 program [8] in $P22_12_1$ using direct methods strategies (TREF 180). Two independent molecules were localized per asymmetric unit.

Anisotropic least-squares refinements were made on 30 of the total 36 non-H-atoms; they were carried out on the SHELX-76 program [9]. H-atoms were partly localized from final ΔF maps and partly calculated. The refinements were stopped when Δ/σ was less than 0.1 for all non-H-atoms. Scattering factors for neutral atoms were taken from *Cromer et al.* [10], except those for H-atoms, which are from *Steward et al.* [11]. *Fig. 6* shows an ORTEP plot of **5**. Fractional coordinates are deposited in the *Cambridge Crystallographic Data Base*. Structure factors, individual bond lengths, and bond angles are available from *M.Z.*

It is very likely that sambucinic acid (**5**) possesses the same absolute configuration as compounds **1–4**, because they are isolated from the same strain of the microorganism. Although the metabolites **1–5** exhibit different structures, they must be interrelated biogenetically. The biogenetic formation of sambucinol (**2**), sambucoin (**3**), and 'apotrichothecene' (**4**) can readily be rationalized assuming that they are derived from trichodiene (**7**) [3]. Compound **7** is a well-established intermediate in the biosynthesis of the normal trichothecenes [6][7]. It is reasonable to assume that also the biogenetic pathway of **5** branches off from **7** as shown in the *Scheme:*

Scheme



Protonation of the exocyclic double bond of **7** leads to the carbenium ion **8**. By cyclization of the latter to cation **9** and subsequent stabilisation, the olefin **10** is formed. Hydroxylation at C(5) and oxidation will complete the formation of **5**.

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REFERENCES

[1] J.W. Jarozewski, P. Grossen, P. Mohr, Ch. Tamm, *Helv. Chim. Acta* **1986**, *69*, 718.
[2] see ref. [2] in [3].
[3] P. Mohr, Ch. Tamm, W. Zürcher, M. Zehnder, *Helv. Chim. Acta* **1984**, *67*, 406.
[4] W.O. Godfredsen, J.F. Grove, Ch. Tamm, *Helv. Chim. Acta* **1967**, *50*, 1666.
[5] J.C. Young, B.A. Blackwell, J. ApSimon, *Tetrahedron Lett.* **1986**, *27*, 1022; J.W. ApSimon, B. Blackwell, R. Greenhalgh, R. Meier, D. Miller, J.R.J. Pare, A. Taylor, *Bioact. Mol.* **1986**, *1*, 125; B. Greenhalgh, B.A.R.J. Pare, J.D. Miller, D. Levandier, R.M. Meier, A. Taylor, J.D. ApSimon, *Bioact. Mol.* **1986**, *1*, 137.
[6] a) Ch. Tamm, W. Breitenstein, in 'The Biosynthesis of Mycotoxines', Ed. P.S. Steyn, Academic Press, New York, 1980, p. 69; b) G.A. Cordell, *Chem. Rev.* **1976**, *76*, 425.
[7] D.E. Cane, S. Swanson, P.P. Murthy, *J. Am. Chem. Soc.* **1981**, *103*, 2136.
[8] G.M. Sheldrick, Universität Göttingen, **1986**.
[9] G.M. Sheldrick, University of Cambridge, **1976**.
[10] D.T. Cromer, J.B. Mann, *Acta Crystallogr., Sect. A* **1968**, *24*, 321; D.T. Cromer, D. Libermann, *J. Chem. Phys.* **1970**, *53*, 1891.
[11] R.F. Stewart, E.R. Davidson, W.T. Simpson, *J. Chem. Phys.* **1965**, *42*, 3175.